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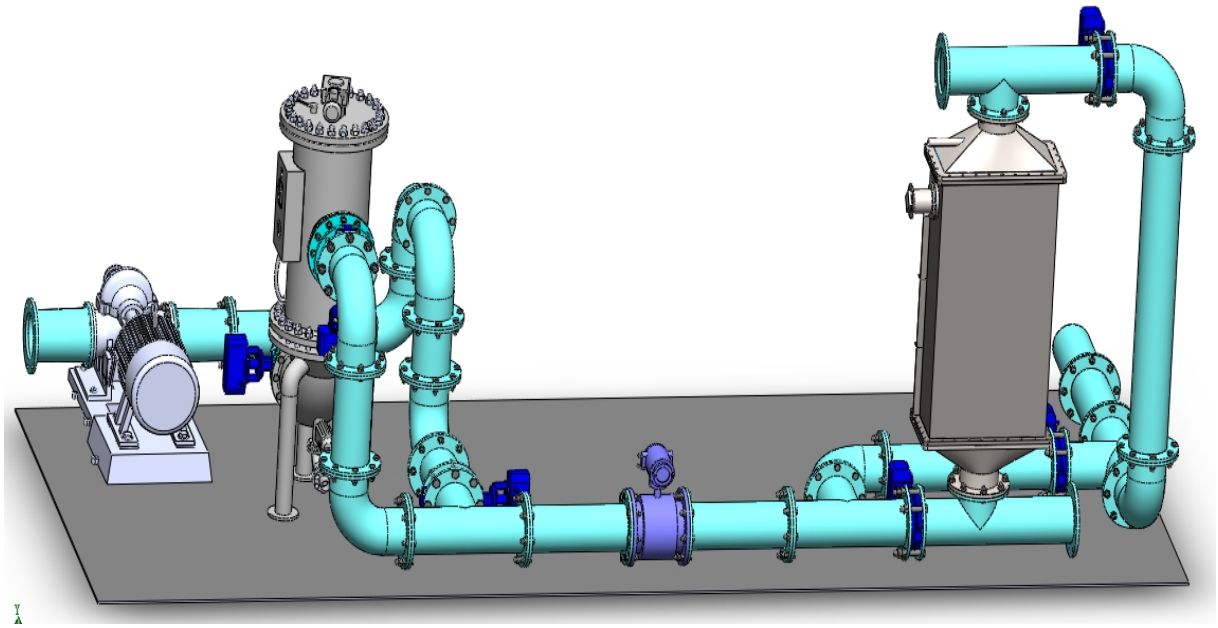
Land based testing of the OceanGuard™ Ballast Water Management System of Qingdao Headway Technology - Final Report



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Abstract

Land-based testing of the Ballast Water Management system OceanGuard™ of Qingdao Headway Technology (QHT) was completed in the period of July 2009 to October 2009. The testing has been conducted according to the IMOs *Guidelines for approval of ballast water management systems (G8), Res. MEPC 174(58) Annex 4* and *Procedure for approval of ballast water management systems that make use of active substances (G9) Res. MEPC 169(57) Annex 1*. The tests were carried out at NIVA's test site located at Solbergstrand 20 km south of Oslo.

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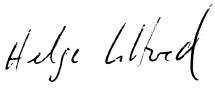

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Contents

Abbreviations and acronyms

APHA – American Public Health Association
A. franciscana – Artemia franciscana
AOX – Adsorbable organic halogens
BWMS – Ballast Water Management System
CFDA-AM - 5-carboxyfluorescein diacetate acetoxymethyl ester
COD – chemical oxygen demand
CT2 – storage tank for control water (5 day)
DBP – disinfection byproducts
DNV – Det Norske Veritas
DO – dissolved oxygen
DOC – dissolved organic carbon
DQIs – data quality indicators
EC_{50}, EC_{10} - the concentrations causing 50 and 10 % effect, respectively, on the test organism
E. coli – Escherichia coli
EOX – Extractable organic halogens
EPA – Environmental Protection Agency (US)
FNU – Formazine Nephelometric Units
GC- gas chromatography
GF/F – glass fiber filter grade F
GLP – Good laboratory Practice
HAA- Haloacetic acids
IMO – International Maritime Organization
ISO – International Organisation for Standardization
LC_{50} – the concentration causing 50 % mortality of the test organism
LEL – Lower Explosive Limit
LLE – Liquid-Liquid Extraction
LOQ- limit of quantification
MSD - mass spectrometry detection
n – number of measurements; in calculating the standard deviation
NDIR - Nondispersive Infrared
NIVA – Norwegian Institute for Water Research
NS-EN ISO – Norwegian, European and International Standard
NTU – Nephelometric Turbidity Unit
OECD – Organisation for economic Co-operation and Development
PAR – photosynthetic active radiation
POC – particulate organic carbon
PSU – Practical Salinity Unit (= ‰)
QAPP – quality assurance project plan
QA/QC – quality assurance/quality control
QHT – Qingdao Headway Technology
QMP – quality management plan
S1-S5 – sampling points 1-5
SPE – solid phase exctraction
Std – standard deviation
TCBS - Thiosulphate citrate bile salt agar
T. suecica – Tetraselmis suecica
THM – trihalomethanes
TOC - Total organic carbon
TRO - Total residual oxidants
TSS – total suspended solids

TT1 – Tank for collection of deballasted treated water
TT2 – storage tank for treated water (5 day)/Tank for collection of deballasted control water
WST – tank with influent prepared test water
X_i - individual analytical result; in calculating the standard deviation
X – the arithmetic mean of individual analytical results; in calculating the standard deviation

Summary

Land-based testing of the OceanGuard™ ballast water management system of QHT has been completed in the period of July 2009 to October 2009. The testing has been conducted according to the IMOs *Guidelines for approval of ballast water management systems (G8), Res. MEPC 174(58) Annex 4* and *Procedure for approval of ballast water management systems that make use of active substances (G9) Res. MEPC 169(57) Annex 1*. The tests were carried out at NIVA's test site located at Solbergstrand 20 km south of Oslo, with medium salinity and high salinity test water. A total of 13 test cycles have been completed. Each cycle lasted for a period of 5 days.

The test water was prepared in a common 516 m^3 tank (WST). A combination of indigenous harvested organisms and cultured surrogate species (>50 µm group: *Artemia fransiscana*; 10-50 µm group: *Tetraselmis suecica*) were added to the test water. *Tetraselmis suecica* is approximately 10 µm in minimum dimension and is regarded as representative of the 10-50 µm group of organisms. This organism is considered to be a relatively robust organism. In addition, a natural collection of harvested organisms were added to fulfil the water quality requirement with regards to the 10-50 µm group and >50 µm group.

Each test cycle included consecutive filtration, ultrasonic and electrocatalysis treatment of >200m^3 test water by the QHT BWMS during transferring of the test water from tank with prepared test water (WST) to a ballast tank (TT2). The treated water was stored in TT2. After 5 days the water was pumped from TT2 to TT1 for sampling before discharge. During pumping, the test water was treated with the ultrasound unit only of the QHT BWMS. The control cycle included transferring >200m^3 of the same type of prepared test water from WST to a ballast tank (CT2) using the same pump, but in by-pass of the treatment unit (QHT BWMS). Control water was stored in CT2 for five days. After the storage period, the control water was pumped to TT2 for sampling before discharge to impose the same pumping influence as the treated water. It was observed a technical failure in auxiliary equipment (air compressor for filter operation) during test cycle 2, which may have influenced the treatment efficiency and the biological data with exception of the toxicity data retrieved from this cycle, as the concentration of active substance was not influenced by the technical failure. This cycle should not be included in the biological efficiency evaluation of the BWMS.

Fulfilment of the chemical and biological requirements of the test water

- The required levels for total suspended solids (TSS), dissolved organic carbon (DOC) and particulate organic carbon (POC) were fulfilled in all 13 test cycles.
- The requirements regarding influent density of the ≥50 µm group was met in all 13 test cycles, except in test cycle 2 were the count was slightly lower than required. In this test cycle, samples for the ≥50 µm group were collected from the surface instead of from the bulk of the tank, resulting in too low counts explained by sinking and swimming activities of *Artemia*. According to the amount of *Artemia* added, the average number per m^3 should be in compliance with G8. The requirements regarding the biological diversity within the population were fulfilled in all tests.
- The requirements regarding influent density of the ≥10-50 µm group was met in all tests, for all three methods used for quantification, with the exception of one of the methods in one of the cycles. The requirements regarding the biological diversity within the population were fulfilled in all tests.
- The requirement regarding the concentration of heterotrophic bacteria in the influent water (≥10^4 CFU ml^{-1}) was fulfilled in all tests.

Biocidal effects of treatment and storage

- The required less than 10 viable organism ≥50 µm in minimum diameter per m^3 in the treated water after five days storage was fulfilled in all 13 test cycles.

- The required less then 10 viable organisms ≥10-50 μm in minimum diameter per ml in the treated water after 5 days storage was fulfilled in all 13 test cycles by using the CFDA microscope method and in 11 of 13 test cycles by using the serial dilution growth method. In the two test cycles (test cycle 2 and 4) exceeding the required limit, the number of organisms were estimated to 24 and 11.6 individuals, respectively, which at least for the latter is very close to the required level. It was estimated to be 8 individuals of the *Cryptomonas* group (size 9-12 μm) in treated water at day 5 in this test cycle. In test cycle 2 there was a technical failure which might have influenced the biological treatment efficacy.
- The equivalent requirement of non-treated control water stating that the level of viable organisms after 5 days of storage should be higher than 100 per ml, was fulfilled in all test cycles, except for test cycle 1 where the number of viable organisms was slightly below the limit for the ≥10-50 μm group.
- Regulation D-2 requires documentation of maximum allowable effluent concentrations after 5 days storage of *Eschericia coli*, *Vibrio cholera* (serotypes O1 and O139) and Intestinal *enterococci,* being <250 cfu/100 ml, <1 cfu/100 ml and <100 cfu/100 ml, respectively. These requirements were fulfilled in all 13 test cycles.

Total residual oxidants (TRO)
TRO was measured as free and total chlorine (mg/l Cl_2) in prepared test water, treated water and control water on day 0 (after ballasting) and after storage at day 1, day 2 and day 5. Total residual oxidants (TRO) in the discharge water of the QHT BWMS at day 5 were below 0.07 mg Cl_2/l for all brackish water test cycles and below 0.09 mg Cl_2/l for all seawater test cycles, and thereby below the recommendations by GESAMP stating that discharge water should not have TRO levels higher than 0.1 – 0.2 mg/l (MEPC 58/2-8 GESAMP BWWG 7/9 Annex 4, 9.3.1).

Disinfection byproducts (DBP)
In the present study, treated water in test cycles 1, 4 and 5 for brakish water, and test cycles 6, 9 and 10 for seawater were sampled and analysed for adsorbable organic halogens (AOX), extractable organic halogens (EOX), bromate, trihalomethanes (THMs) and other halogenated organic and inorganic compounds. GESAMP issued a document specifying the DBPs to be analysed (GESAMP-BWWG (MEPC 59/2/13 March 2009). Compounds listed by GESAMP have been analysed in the present tests. The dominating halogenated organic compound, and THM, was bromoform. After deballasting on day 5, bromoform was detected in concentrations in the range from 290-670μg/l for brackish water and 120-170μg/l for seawater. The dominating non organic by-product was bromate. Bromate was found in concentrations up to 6.6μg/l in brackish water and up to 1.9μg/l in seawater after deballasting on day 5. The concentrations of the sum parameters AOX and EOX were low compared to the concentrations of bromoform in the same test cycle.

Toxicity
A total of 45 toxicity tests with 6 different species and 5 different phyla have been performed. In the growth inhibition tests with algae with discharge water on day 5, effect was only observed in one out of 13 tests. In acute and chronic tests with juvenile turbot (*Scopthalmus maximus*), no observations of toxic effects were observed. In the invertebrate tests performed using the copepod *Acartia tonsa*, no toxic effects were observed in any of the tests. The reproduction of *B. plicatilis* was tested both in brackish water and seawater at 5 concentrations of treated ballast water after discharge. Statistical assessment of the reproduction indicated no significant effects. In the oyster larvae test, the observed toxic effects on the larvae in treated ballast water in two test cycles could not be claimed to be caused by the BWMS, since the toxic effects in untreated waters was higher, possible due to toxic effects of remaining bacteria.

The results of the toxicity testing indicate that treatment with QHT BWMS produce ballast water with little or no toxic effects upon discharge. It is therefore unlikely that the treated and discharged ballast water will have any adverse effect in the recipient water upon deballasting.

1. Background

The goal for QHT is certification of their BWMS in accordance with the requirements in the IMO Convention on ballast water management (IMO, 2004) and the underlying guidelines; *Guidelines for approval of ballast water management systems (G8), MEPC 58/23/, Annex 4, Res. MEPC 174 (58), 2008* and *Procedure for approval of ballast water management systems that make use of active substances (G9) MEPC 57/21 Annex 1, Res. MEPC 169 (57), 2008,* section 1-5, pages 1-7. Guidelines are hereafter referred to as G8 and G9.

Land-based testing for type approval, as reported here, was conducted in the period of 22ndof July 2009 to 26th of October 2009. The tests were conducted in accordance with G8 and G9.

2. Materials and test protocols

2.1 Test site

The tests were conducted at NIVA's test site located at Solbergstrand 20 km south of Oslo. Seawater was supplied from various depths down to 60 m in the Oslofjord, while fresh water was supplied from ground water bore holes or from a local creek.

The test facility includes 4 glass-fibre reinforced polyester tanks, supplied with inlet and outlet arrangements and equipment for proper cleaning. During storage of treated and control water, the tanks are covered to prevent light introduction (TT1, TT2 and CT2). The surfaces of the tanks are coated with coatings for ships (Balloxy HB light, Jotun, Norway). Propeller devices with gentle rotation are mounted at the bottom and at shallow depth in WST and TT1 (if necessary as judged by measurements of homogeneity), and at the bottom in TT2 and CT2. These propeller devices are used to suspend particles, including algae and zooplankton, evenly and homogenise the content. A measurement series is conducted to verify homogenisation prior to each test and prior to sampling.

2.2 The evaluated ballast water treatment system

The evaluated OceanGuard^{TM} ballast water treatment system was supplied by QHT. This technology uses the advanced Electrocatalysis enhanced by Ultrasonic Technology (EUT) to achieve the online ballast water treatment. Water is pre-treated by a 50 μm filter before the EUT unit during ballasting. For deballasting, the water is by-passed the filter and the electrocatalysis unit, but treated by the ultrasonic unit.

2.2.1 General description of the QHT BWMS as described by the vendor

The following description in chapter 2.2.1. is solely the product of the vendor and has not been subject to verification by NIVA.

Small-scale testing was carried out by NIVA in January 2009. The system prepared for the land based tests at NIVA was installed in the 20 feet ISO container. The major system components are ballast pump, filter and EUT unit, flow meter for ballast water line, and the salinity and TRO sensor for automatic control of the system. The power supply for ballast pump, filter, and EUT unit is ensured by the use of the Solbergstarnd grid power supply and remote control panel with the data acquisition system of operational and monitoring data of the system is installed inside of the container.
The main components of OceanGuard™ Ballast Water Mangement System are as following (description by QHT):

Control unit

Control unit consists of control system, recording system, display system and alarm system. It is in charge of the entire system controlling, including the processing of every monitoring signal, the alarm signal, the linkage of the system and the auto-control of the system startup and shutdown order. This unit contains all the necessary controlling procedures in this system, and it displays the operating conditions of the control system which includes the operating conditions of every component. If the system breaks down, the control unit will make audio and light alarm, at the same time, power will be cut off automatically, or, some troubleshoot can be realized automatically by executing the related process. For the operating condition of the device, it will be recorded and memorized, and the control unit will display the data according to the formal test requirements. Meanwhile the operators can take control and adjustment of the system through the control unit, and its control panel can realize the bidirectional interlock control both in short and remote distance. It is not only convenient for on-site operation but also for remote operation. The whole process is convenient, safe and reliable.

Filter

A full-automatic back flushing filter with the filtration degree of 50μm is set before the EUT unit and it can remove the organisms and other impurities with the diameters larger than 50μm. This filter will start backwashing automatically without any artificial operations when the pressure loss exceeds the set value. The filter works during ballasting only.

EUT Unit (Power Supply and Treatment Unit)
EUT unit is composed of two parts: the electrocatalysis part and ultrasonic part. In the electrocatalysis part, special semiconductor catalyzing material of high performance is adopted, which can generate a great deal of active substances like hydroxyl radical. This part is of high current efficiency with a relatively long lifespan. This part can remove most of the organisms and bacteria. For the ultrasonic part, it's a popular technology at present, as ultrasound can make high pressure instantly in partial areas and strike the cell of organisms in very short time. As a supplementary part of the electroatalysis device, this can further improve the sterilization effects.

Sensor
Sensors include salinity meter, flow meter and TRO sensor, and they can respectively measure the parameters of salinity, flow rate and TRO, in order to accurately reflect the operating status of the system in time. Adjustment will be made according to the data of sensors by the control unit for an ideal treatment effect. Salinity, flow rate and TRO are important parameters in the control process, and through calling the internal store program, the control unit can make EUT unit proceed with the relevant initial operating mode and optimum TRO operating status.

2.3 Test waters

Test waters were prepared in a 516 m^3 tank (WST) from high salinity sea water from 60 meters depth or from brackish surface water depending on the required salinity (>32 PSU or 3-32 PSU, respectively, with a minimum difference of 10 PSU). In the lower salinity range 22 PSU is envisaged. If necessary, fresh water from a surface water source or groundwater wells was added to adjust the salinity. The 516 m^3 of test water was used for both testing and control. A combination of harvested indigenous organisms and cultured surrogate species (≥50 μm: *Artemia franciscana* ; 10-50 μm: *Tetraselmis suecica;* if necessary heterotrophic bacteria) were added to fulfil the biological water quality criteria (see Table 2), and freshwater, soluble lignin, starch and kaolin were added to adjust the salinity and the contents of dissolved organic carbon (DOC), particulate organic carbon (POC) and total suspended solids (TSS), respectively, to within the limits of chemical water quality criteria (see Table 1). At least 5 test cycles with each of the two water qualities (high and medium salinity; test water 1 and test water 2 in table 1) were conducted. Each test cycle took place over a period of 5 days.

2.3.1 Overview of requirements

Table 1 summarizes the chemical requirements of the test waters specified in G8, while **Table 2** summarizes the biological requirements.

Table 1. Required chemical water quality of test waters. Salinities should be separated by at least 10 PSU.

	Salinity	**DOC**	**POC**	**TSS**
Test water 1	>32 PSU	>1 mg/l	>1 mg/l	>1 mg/l
Test water 2	3-32 PSU	>5 mg/l	>5 mg/l	>50 mg/l

Table 2. Required biological water quality in influent test water, treated water and in control water after 5 days storage as stated in regulation G8 by IMO.

Organism group	Influent water	In treated water after 5 days storage (Regulation D-2)	In control after 5 days storage**
≥50 µm min. dimension	Pref. 10^6 m^{-3}, ≥10^5 m^{-3} Min. 5 species from 3 diff. phyla/divisions	<10 viable organisms per m^3	> 100 viable organisms per m^3
≥10-50 µm min. dimension	Pref.10^4 ml^{-1}, ≥10^3 ml^{-1} Min. 5 species from 3 diff. phyla/divisions	<10 viable organisms per ml	> 100 viable organisms per ml
Heterotrophic bacteria	≥10^4 cfu ml^{-1}	-	-
Vibrio cholerae	-	<1 cfu/100 ml	-
Escherichia coli	-	<250 cfu/100 ml	-
Intestinal *Enterococci*	-	<100 cfu/100 ml	-

** As discussed in the results and discussion section.

2.3.2 Assurance of fulfilment of chemical water quality test criteria

The water quality with regards to chemical criteria was prepared prior to addition of organisms and treatment of the water in the treatment system. The 516 m^3 tank was filled with water from the appropriate source. Rapid quantification test kits (HACH methods 10129 and 8006) can, but will not necessarily be used to estimate concentrations of DOC, POC and TSS, respectively, in the test waters on site. The amount of soluble lignin, starch and kaolin to be added to the test waters was calculated from measured values and the known concentrations of the respective stock solutions. Additionally samples were taken and analysed by standard methods for DOC, TOC (POC) and TSS at an accredited laboratory. The samples were taken from the top of the water column in the tank, after ensuring homogeneity in the tank. Before sampling, all water contents were homogenized as described in chapter 3.1.

Any necessary adjustment to the chemical water quality was made before any additional organisms were added to the test water to minimize stress on the organisms.

2.3.3 Assurance of fulfilment of biological water quality test criteria

A combination of indigenous harvested organisms and cultured surrogate species (>50 µm: *Artemia franciscana*; 10-50 µm: *Tetraselmis suecica*) were added to fulfil the biological water quality criteria described in **Table 2**. Measurements by NIVA shows that *Tetraselmis suecica* has an average minimum diameter of 9.3 µm (n=25) when growing exponentially in our cultures, the diameter range is 7-11 µm. *T. suecica* is quite robust and has an outer shell composed of cellulose-like material. It has a good survival when exposed to shear forces in pumps and a good survival in the dark. In addition it is quite tolerant with respect to survival in brackish seawater. *T. suecica* is therefore a robust representative of the type of organisms to be expected in the 10-50 µm size fractions of marine organisms.

Cultivation of Artemia franciscana and Tetraselmis suecica

The criteria of minimum concentration of organisms ≥50 µm in minimum dimension was fulfilled by adding cultured *Artemia franciscana*, and the equivalent criteria for organisms ≥10-50 µm in minimum dimension was fulfilled by adding cultured *T. suecica*.

A. franciscana: Resting cysts of *Artemia franciscana* are available commercially. Hatching of cysts were achieved by adding approximately 0.1 g of cysts to 1 litre of 20 ‰ salinity seawater. The culture was incubated with a bright light source at a temperature of 22-26 °C with good aeration in the

medium. Full hatching is usually achieved within 48 hours. It is possible to hatch approximately 100 000 nauplii per litre. *Artemia* nauplii are hatched with a supply of food (egg yolk) and will therefore live for up to a week without any external food supply. Survival length is twice that if the nauplii is stored at 8-10 °C. However, nauplii should be used within 2-3 days in order to achieve high survival and viability.
T. suecica: The algae were grown autotrophically in seawater growth media with added nutrients. The seawater was disinfected by filter (200µm, 50µm and 0.2µm) and UV radiation before use. Algae culture method used is the "growing culture volume" technique from isolated algae strains which are always available from NIVA's algae culture collection. Large volume cultures need gentle aeration in order to maintain satisfying oxygen levels, bright light and controlled temperature. Densities close to 10^9 per 100 ml have been reached after 7 days pre-culture and 7 days tank culture.
The necessary cultivation volume was calculated based on a final density (10^5 nauplii per litre for *A. franciscana* and 10^7 per ml for *T. suecica and*, final volume of the test water 500 m^3) and the desired concentration of the organism in the test water (10^5 per m^3 for *A. franciscana* and 10^3 per ml for *T. suecica.*).

Harvesting of indigenous organisms

Harvesting of indigenous algae and planktonic animal species, using a Unik Filter type 450 (Unik Filtersystems, Os, Norway) equipped with a 20 µm mesh size screen, was done to assure the fulfilment of the criteria regarding at least 5 species from 3 different phyla/divisions of both test groups of organisms ≥10 µm in minimum dimensions. The harvesting process has been shown to be relatively gentle to the organisms; surface water (1 m depth) is smoothly pumped (ca. 3 m^3/h) by a jet-pump to the inlet side of the screen, and algae and animals are washed from the screen to a collecting tray (ca. 100 l/h, e.g. approx. 30x conc.) and transported to a storage tank. The transport to the storage tank and from the storage tank to test-tanks was as gentle as possible with a minimum of pumping. The variability (phyla, species), viability and general conditions of harvested organisms were evaluated by microscopy to ensure that the test criteria most probably will be fulfilled.

Bacteria

The concentration of heterotrophic bacteria in the brackish testwater is normaly exceeding the required concentration of $\geq 10^4$ CFU ml^{-1}, while the heterotrophic bacteria criteria for the high salinity test water is expected to be fulfilled by the heterotrophic communities accompanying the cultured *Tetraselmis suecic* and *Artemia franciscana* . If necessary, extra heterotrophic bacteria can be added as cultivated organisms. A pre-culture of marine bacteria is then added to nutrient broth and cultivated at room temperature for two days before added to the test water.

Discharge of water

The potential environmental impact of discharge of test and control waters, as well as sludge from filtering treatment was evaluated. In the case of adverse effects, measures will be taken to counteract the adverse effects by treating the waters prior to discharge. Discharge permit from the Norwegian Pollution Control Authority represented by the County Governor in Oslo and Akershus has been obtained.

2.4 Test procedure

2.4.1 Description of test cycle

The different water transfers between tanks via the OceanGuard™ BWMS during a test cycle that includs a treatment cycle and a control cycle is shown in Figure 8.

One treatment cycle (blue lines) involved pumping of >200 m^3 test water from the storage tank WST to the OceanGuard™ BWMS for full treatment (filtration, electrocatalysis and ultrasonication) and

storing the treated water in TT2 for 5 days. During deballasting the water was pumped through the ultrasonic unit of the BWMS, bypassing both the filtration and the electrocatalysis unit to the storage tank TT1.

A control cycle (red lines) involved pumping of >200 m^3 of the remaining batch of prepared test water from WST to the storage tank CT2 using the pump of the BWMS. The control water was stored in CT2 for 5 days before deballasting to the holding tank TT2. Prior to transfer of control water to TT2, this tank was rinsed with freshwater to remove traces of treated water and active substance remaining after the storage of treated test water.

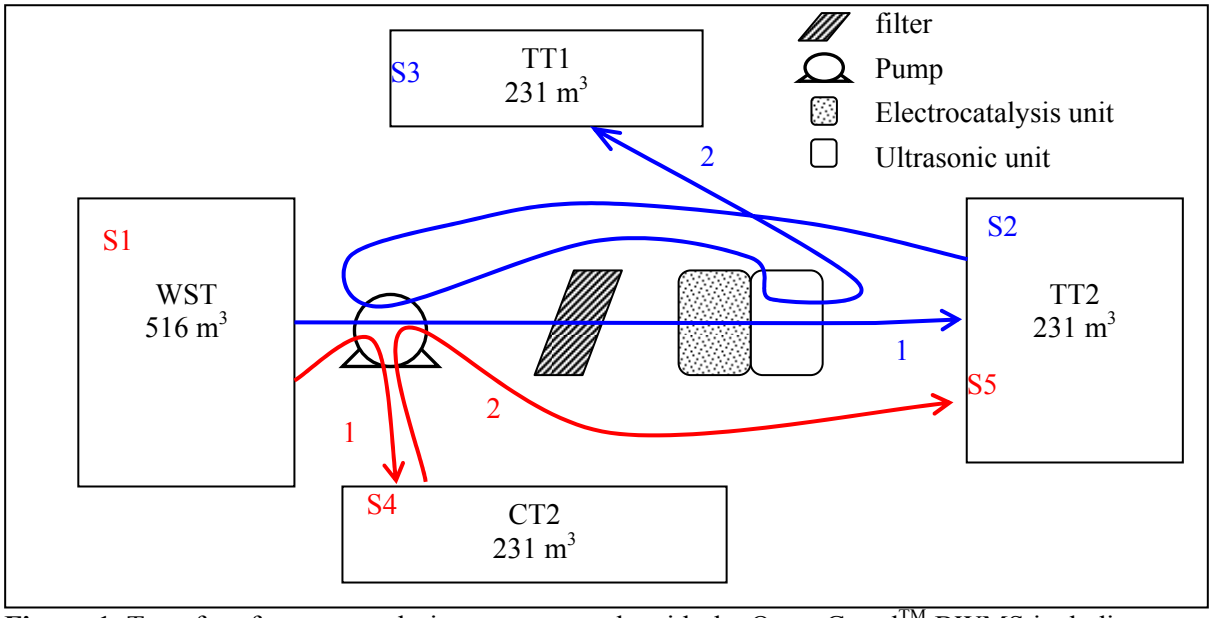

Figure 1. Transfer of test water during one test cycle with the OceanGuard™ BWMS including a test line (blue) and a control line (red). Blue line 1 indicates the day 0 (ballasting) operation of treated water, whilst blue line 2 indicates the day 5 (deballasting) operation of treated water. Red line 1 indicates the day 0 (ballasting) operation of control water. Red line 2 indicates the day 5 (deballasting) operation of control water. Sampling numbers are indicated by S1-S5 (red).

2.4.2 Measures to avoid cross-contamination during water transfer and sampling

To avoid cross-contamination between consecutive test waters upon transfer between tanks, all pipelines and tanks were flushed for 2-3 min with sea water from 60 meters depth or ground water with documented quality between each test cycle, followed by rinsing with high temperature water (80-90°C).

The same holding tank (TT2) was used for both treated water and control water in the same test cycle. To avoid contamination, treated water was always introduced to TT2 before control water. No cross-contamination was therefore possible to occur because the density of organisms was much lower in treated water than in control water. To avoid cross-contamination during sampling, the buckets, siphon overflow and plankton net were rinsed in sea water from 60 meters between each sampling.

2.5 Sampling

2.5.1 Assuring the representativeness of samples

Before any samples are collected the following procedure is carried out to assure that representative samples are withdrawn:

1. The water is homogenized using a propeller device before sampling from WST, and in TT2 and CT2 after day 0 ballasting operation and from TT1 and TT2 after 5 days storage and deballasting operation. The turbidity in different sections of each tank (upper part, middle part and bottom part, in both periphery and center) is then measured by a handheld submersible probe (YSI – 600 OMS) during homogenization. When any turbidity measurement is within a 10 % deviation from the average turbidity of all measurements in the tank, sampling is started.
2. The particulate content in the water is kept in suspension using propeller devices. Propeller devices are in operation between transfer of water from WST to TT2 and CT2, and before transfer from TT2 to TT1 and CT2 to TT2. The particulate content in TT2 and TT1 is also kept in suspension after transfer, using propeller devices. The turbidity in different sections of each tank (upper part, middle part and bottom part, in both periphery and center) is then measured as described above.

2.5.2 Sampling protocols

The following procedures were used to collect samples from the different tanks and sampling times. All sampling were done in triplicates.

In WST (S1), TT2 (S2), TT1 (S3), CT2 (S4) and TT2 (S5):

1) *Sampling of bacteria*: Bacterial samples are collected as 3x 1000 ml grab samples by slowly submerging a 1000-ml sterile bottle with thiosulfate. The bottle is closed immediately after sampling and cooled at 4°C.
2) *Sampling of organisms ≥ 50 µm in WST (S1), CT2 (S4) and TT2 (S5).* A plastic bucket is used to collect 3x 20-100 litre sample. The sampled water is slowly sieved through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup.
3) *Sampling of organisms ≥ 50 µm in TT2(S2) and TT1 (S3):* A siphon spillway is used to collect 3x 1 m^3 test water from TT1 directly after transfer, and from TT2 after 5 days storage. The water is sieved directly through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup. The sieved water is collected in known volume tanks to ensure accurate sampling volume.
4) *Sampling of organisms 10-50 µm*: Organisms with a minimum diameter between 10 µm and 50 µm are sampled as 3x 1000 ml for control water with clean glass bottle and 3x 10 l for treated water with a clean plast bucket.
5) *Sampling of test water for pH, salinity, organic carbon measurement*: water samples are collected as 3x 1000 ml grab samples by slowly submerging a 1000-ml clean plastic bottle. The bottle is closed immediately after sampling.
6) *Sampling of test water for TRO measurement:* water samples are collected as 1000 ml grab samples by slowly submerging a 1000-ml clean glass bottle pretreated with bleach to remove any chlorine demanding substances. TRO is measured immediately after sampling. See intern procedure.
7) *Sampling of test water for DBP measurement (decay test):* water samples are collected as 6x 1000 ml samples by slowly submerging a 1000-ml ALS-glass bottle with 150mg/L thiosulfate. The bottle is top filled, closed immediately and cooled at 4°C.
8) *Sampling of test water for acute, chronic and sub-chronic toxicity test:* water samples are directly collected as 1x 1000 ml grab samples by slowly submerging a 1000-ml clean glass bottle. The bottle is closed immediately after sampling. For fish toxicity test, more than 300l test water is directly collected in stainless steel container from which the fish pool is supplied.
9) *Sludge samples:* Sludge from the filter backflush water was sampled two times during the testing programme as 2x 1000ml grab samples.

2.5.3 Overview of sampling equipment

An overview of sampling equipment, containers used and sampled volumes are given in **Table 3**.

Table 3. Equipment and containers used for sampling and necessary sample volume for the individual parameters.

Parameter	Sampling equipment	Sample container	Collected volume for Influent and control water	Collected volume for Treated water
Turbidity	Turbidimeter submersible probe	-	-	-
pH	pH probe	-	-	-
Temperature	Temp. meter	-	-	-
Salinity	Probe			
Dissolved oxygen	DO probe	-	-	-
Redox	Probe	-	-	-
DOC**	Directly			
POC**	Directly			
TSS	Directly	Clean plastic bottle	3x 1000 ml	3x 1000 ml
Disinfection by-products and chemical fate analysis (DBP)	Directly	ALS laboratory glass bottle	6x 1000 ml	6x 1000 ml
Total residual oxidants (TRO)	Directly	Clean glass bottle pretreated with bleach to remove any chlorine demanding substances	1x 1000 ml	1x 1000 ml
Sludge characterization	Directly from filter backflush	Clean plastic bottle	-	1x 2000 ml
Organisms ≥ 50 µm	Sieving*	Clean glass bottle	3x 20 – 100 l	3x 1 m³
Organisms 10-50 µm	Directly	Clean glass bottle, clean plastic bucket	3x 1000 ml	3x 10l
Heterotrophic bacteria				
Coliform bacteria, *E. coli*				
Enterococcus group bacteria	Directly	Sterile bottle with thiosulfate	3x 1000 ml (6x500 ml)	3x 1000 ml (6x500 ml)
Vibrio sp.				
Vibrio cholerae				
Acute/Chronic Algae, Copepods, rotatoria, oyster embryo	Directly	Clean glass bottle	1000 ml	1000 ml
Acute fish, Juvenile fish	Directly	Stainless steel container	300 l	300 l

* A 20-100-litre grab sample (from influent and control water) or 1 m^3 collected through a siphon spillway (from treated water) is concentrated to a volume of 40-100 ml through a plankton net with diagonal dimensions of 50 µm.

** Eventually, depending of the laboratory necessary duty conservation, samples from the 1000 ml bottle is transferred to 100 ml acid washed glass bottles.

2.5.4 Sampling of head gas

The head gass measurements performed during operation and storage of treated ballast water were done to assure the safety of test personell and equipment. Hence, both the analytical equipment and gass collection was designed with this in mind. Head gass was measured in the manhole in the headspace of each tank from the beginning to the end of the pumping (S2, S3, S4, S5). Gas was measured using gas sensors:

- Drager x-am 5000: Cl_2 and flammable gases. The gas sensor for flammable gases will measure all flammable gas at 65°C, including both methane and hydrogen.
- GasAlert MicroClip RW: CO and H_2S.

These gas sensors were mounted inside the lid-covered tanks just inside the manhole.

2.5.5 Sample preservation and transportation

General preservation and handling methods of water samples are described in NS-ISO 5667-3 (2003) water quality- Sampling –Part 3: Guidance on the preservation and handling of water samples and in EN ISO 19458 (2006) for the microbiological analysis. All samples, which have to been sent to a laboratory for analysis, are collected, clearly identified and packed in a cooler bag (4^{o}C) under transport. When the samples arrived at the laboratory, they were stored in a cool room. The samples for organic carbon measurements were conserved with 1ml H_2SO_4 per 100 ml of sample (pH<2) and cooled at 4^{o}C until the samples is analysed. The samples for disinfection by-products measurements were conserved with thiosulfate. All details for transport and storage of samples were clearly specified with the sub-contractor laboratories which were responsible for it (Appendix N). Preservation methods and expected storage/holding times before measurement are shown in Table 4.

Table 4. Preservation methods and expected storage/holding times before measurement (ISO/CD 5667-3, 2001).

Parameter	Preservation	Maximum holding time	Expected storage time
Temperature	-	-	0
pH	-	-	0
Dissolved oxygen	-	-	0
Salinity	-	-	0
Turbidity	-	-	0 (probe) 0-24 hours (lab)
Redox	-	-	0
TRO	-	-	0
Disinfection by-products (DBP)	Neutralisation with thiosulfate. Stored in dark, 4°C, top-filled bottles	7 days	0-7 days
Dissolved organic carbon (DOC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml (pH<2), 4°C	7 days	0-5 days
Total organic carbon (TOC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml(pH<2), 4°C	7 days	0-5 days
Particulate organic carbon (POC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml(pH<2), 4°C	7 days	0-5 days
Total suspended solids (TSS)	4°C	24 hours	<24 hours
Organisms ≥ 50 µm	4°C	6 hours	< 2 h
Organisms 10-50 µm	4°C	24 hours	< 24 h
Heterotrophic bacteria	With thiosulfate, 4°C	24 hours	< 24 h
Coliform bacteria, *E. coli*			
Enterococcus group bacteria			
Vibrio sp.			
Vibrio cholerae			
Acute/Chronic Algae, Copepods, rotatoria, oyster embryo	4°C	24 hours	< 24 h
Acute fish, Juvenile fish	16°C	24 hours	< 24 h
H_2S	-	-	0
Flammable gases (LEL)	-	-	0
CO	-	-	0
Cl_2	-	-	0

2.6 Analyses

2.6.1 In situ measurements

Temperature

Temperature was measured *in situ* using a calibrated thermometer. Temperature is reported in ºC.

pH

pH was measured *in situ* using a calibrated probe and pH-meter.

Dissolved oxygen (DO)

Dissolved oxygen (DO) was measured *in situ* using a calibrated probe and meter. DO is reported as mg O_2/l.

Salinity

Salinity is measured *in situ* using a calibrated salioterm. Salinity is reported in PSU.

Redox potential

Redox potential will be measured *in situ* using an Orion Redox/ORP electrode (Orion 9678 BNWP, Thermo Electron Corporation). Redox potential is reported in mV.

Gas phase parameters

Drager x-am 5000; flammable gases (% of LEL (Lower Explosive Limit)), Cl_2 [ppm] and GasAlert MicroClip RW; H_2S [ppm] ,CO [ppm] designed to cover concentration ranges potentially harmful to human and ship safety.

2.6.2 Discrete samples

Total residual oxidants (TRO)

Total residual oxidants (TRO) is measured by the colorimetric DPD-method (American Public Health Association, 1989), which is currently the method recommended for measurement of TRO in seawater (Buchan et al., 2005). The method is based on the oxidation of N,N-diethyl-p-phenylendiamin (DPD) which turns to a pink Wurster-cation in the presence of strong oxidants. The intensity of the colour is proportional to the TRO concentration. The colour intensity is measured by a Hach DR/2000 spectrophotometer (Hach Company, Loveland, CO, USA). The method and the instrument give the results as total residual oxidants (TRO) as mg/l Cl_2. TRO are reported as free and total concentration of chlorine. The detection range of this method is 0.02-0.20 mg/l.

Turbidity

Turbidity is measured using a 6035 Turbidimeter (Jenway) with formazin as standard (Formazin Turbidity Standard 4000 NTU, HACH, 2461-42 and reported as Formazin Nephelometric Units (FNU) or as Nephelometric Turbidity Units (NTU).

Dissolved and total organic carbon (DOC and TOC)

DOC and TOC is measured by an accredited method based on Norwegian Standard NS-ISO 8245 (NIVA method G5-3) at NIVA. TOC is measured on the whole sample and DOC is measured after filtering the sample through a GF/F filter (0.7 µm). The sample is acidified with phosphoric sulfuric acid and aerated with oxygen to remove inorganic carbon. The sample is injected in a quartz tube filled with a platinum catalyser at 680 ^oC. The organic carbon compounds are oxidized to CO_2 which is quantified using an NDIR detector (Phoenix 8000 TOC-TC analyser with sample carousel STS 8000) with oxygen as carrier. Detection limit is 0.2 mg C/l.

Particulate organic carbon (POC)

POC is calculated as the difference between the level of TOC in the sample, measured on the non-filtered sample (see 4.2.3), and the measured DOC level of the same sample. POC is also measured at NIVA (method G6) as the amount of organic matter accumulating on a glass fibre filter GF/F (0.7 µm) when a known amount of sample is filtered. The dry sample is encapsulated in tin capsules which are ignited in oxygen saturated heliumgas at 1800 ºC. Surplus oxygen is removed by Cu at 650 ºC and the off-gases are passed through a chromatographic column, where upon CO_2 is detected (Carlo Erba Elementanalysator 1106, with samplechanger AS 400 LS). The method is based on CARLO ERBA ISTRUMENTAZIONE, ELEMENTAL ANALYZER 1106. Instruction manual, APPLICATION LAB REPORTS, Elemental analysis lab, Carlo Erba. January 1987. Detection limit is depending of the volume of sample filtered. For 50-100ml filtered sample, the detection limit is between 0.05 and 0.1 mg C/l.

Total suspended solids (TSS) and ignition loss

TSS is measured at NIVA (method B1/2) in accordance to NS-EN 872 and NS 4733. A glassfiber filter GF/F (0.7 µm) is washed with distilled water, dried at 105 ^{o}C for 30 minutes, then ignited at 480 ^{o}C for 2 hours and finally weighed. The sample is filtered through a filter prepared as described earlier. The filtered samples are dried for 1 hour and weighed. The TSS is represented by the weight increase. Lowest reported value: 0.1 mg/l. The filter with the residue is then ignited at 480 ^{o}C and the ignition loss is determined by weighing.

Settling solids and density

Settling solids is measured at NIVA in accordance with NS-EN 14702-1:2006 Characterisation of sludges - Settling properties - Part 1: Determination of settleability (Determination of the proportion of sludge volume and sludge volume index). Density is determined at NIVA as the weight of a known volume of the sample, divided by the volume of the sample.

Disinfection by-products

Samples are collected in 1 litre ignited glass bottles with pre-added sodium thiosulphate in powder form (150 mg), top-filled, capped and stored at 4ºC in the dark. The samples are shipped to sub-contractor (Appendix N) for analysis within 7 days. In addition, one (1) 2.5 litre ignited glass bottle with pre-added sodium thiosulphate in powder form (375 mg), top-filled, capped and stored at 4ºC in the dark at Solbergstrand as a spare sample for each collected sample.

All possible relevant disinfection by-products are analysed by an external laboratory (Appendix N) including those listed in Table 10 (source from MEPC.57/2/3 and GESAMP-BWWG 4/9).

For each salinity-range test waters:

- Samples collected during the first test cycle will be analysed for all disinfection by-products on prepared test water (S1), test water after ballasting day 0 (S2) and after deballasting day 5 (S3), control water after ballasting day 0 (S4) and after deballasting day 5 (S5).
- For two other cycles, DBPs analyses will only be done on test water after ballasting day 0 (S2) and after deballasting day 5 (S3).
- For one of these test cycles a chemical fate's evaluation will be performed analysing for selected disinfection by-products after two (2) days storage after ballasting treatment, after 5 days storage before deballasting (S2) and in stored effluent samples after deballasting after 30 min, 2 h, 4 h, 24 h and 48 h or similar considering the aquatic toxicity results and the TRO. Also, control water i sampled 0 hours and 48 hours after deballasting.

AOX

Determination of adsorbable organically bound halogens (AOX) is described in DIN EN ISO 9562:2004 by a solid phase extraction (SPE) in waters with high salt content. AOX represents the sum of organically bound chlorine, bromine and iodine (but not fluorine) which can be adsorbed on activated carbon under specified conditions and, if the sample is not filtered, includes that associated with suspended matter.

EOX

Extractable Organically bound halogens (EOX) is measured by solvent extraction with microcolorimetric tittering as described in DIN 38409-H8:1984.

Trihalomethane compounds

Trihalomethane (THM) compounds: trichloromethane (chloroform), bromodichloromethane, dibromochloromethane, tribromomethane (bromoform) will be analysed by the purge and trap method described in DIN EN ISO 15680/ US-EPA 524.2 with a GC-MS detection.

Bromate

Bromate ions are measured by Liquid Ion Chromatography as described in DIN EN ISO 10304-1:1992 Determination of dissolved fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulfate ions, using liquid chromatography of ions. Part 1: Method for water with low contamination.

Haloacetic acids

Haloacetic acids (HAA) is determined by gas chromatography (GC-MS detection) after a liquid-liquid extraction and a derivatization as described in EN ISO 23631/DIN 38407 F25.

Halogenated acetonitrile compounds

Monobromoacetonitrile, dibromoacetonitrile, bromochloroacetonitrile, dichloroacetonitrile and trichloroacetonitrile are analysed by the American US EPA 551.1 method: determination of chlorination disinfection by-products, chlorinated solvents, and halogenated pesticides/herbicides in drinking water by liquid-liquid extraction, derivatisation and gas chromatography with electron-capture detection.

Bromophenol

Bromophenol compounds are measured by gas chromatography with mass spectrometry detection after a liquid-liquid extraction and derivatisation.

Tribromobenzen, chlorotoluene and halogenated aliphates

Tribromobenzene, chlorotoluene and the halogenated aliphates analysis follows also DIN EN ISO 10301-F4/ US EPA 524.2: purge and trap gas chromatography with a mass spectrometry detector.

Characterization of sludge from filter backflushing

In one test cycle for each salinity range the backflush water from the filter is analysed for sludge parameters to be compared to the influent water at ballasting. The parameters measured were:

- pH-value (as described above)
- Salinity as practical salinity units (as described above)
- Dissolved Oxygen (on-site) (as described above)
- Redox potential (as described above)
- Total Suspended Solids (TSS) (as described above)
- Total Organic Carbon (TOC) (as described above)
- Dissolved Organic Carbon (DOC) (as described above)
- Particular Organic Carbon (POC) (as described above)
- Ignition loss (as described above)
- Settling Solids (as described above)
- Density (as described above)
- Turbidity (as described above)

Determination and quantification of organisms ≥50 µm

Organisms >50 µm are inspected in microscope at 10-40x magnification within 6 hours after sampling. Viable organisms are counted and identified based on motility and integrity according to OECD (1985): OECD Test Guideline for Testing of Chemicals 202, "*Daphnia* sp. acute immobilisation test and reproduction test".

Determination and quantification of organisms ≥10-50 µm

The viability of the micro-plankton (≥10-50 µm) was determined by observing cells incubated with 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) according to Ganassin et al. (2000). A

10 ml sample was incubated for 1 hour with 4 µmol of CFDA-AM. The sample was fixed with formalin and filtered onto black polycarbonate filters. The filter was mounted on a glass slide in paraffin oil and frozen. CFDA-AM is hydrolysed only in a living cell. CFDA-AM is a marker for cell membrane integrity and may be measured directly in cells. In principle, the non-fluorescent chemicals CFDA-AM is taken up in the cytosol, where it becomes hydrolysed into fluorescence end products. These end products are trapped inside the cellular compartment and may be observed in an epifluorescence microscope using excitation filter 485 nm and emission filter of 530 nm. In the epifluorescence microscope viable cells are observable as brightly yellow/green coloured cells, while non viable cells are pale green (heterotrophic cells) or pale green with red autofluorescence of the chloroplast (photoautotrophs). Numbers of viable and non viable cells were counted at 300x – 480x magnification.

As a complementary method to direct counting for testing of viability, the serial dilution method in algal growth medium was used. The serial dilution method is often referred to as the most probable number method. It is simply based on the fact that by diluting the sample in a sequence and observing in which dilutions the organisms occur (grow) in afterwards, one is able to backward calculate the number of cells in the original sample. The dilution series were achieved by adding 1 ml of sample to 9 ml of media (algal growth media, 20 % Z8 seawater media). After mixing, 1 ml of this sample was further diluted with 9 ml. In this way a series of 10x dilution were made. The number of dilutions was set to cover the expected cell density range in the original sample. 3-5 parallels were employed in order to provide statistical significance of estimated number.

A supplementary cultivation test was also used by plating on agar plates. 100 µl of samples was spread on a seawater agar growth medium and incubated in constant light for 72 hours at 20 ^{o}C. Colonies of *Tetraselmis sp.* was observed by viewing agar plates in stereo microscope at 160x magnification. The procedure has a detection limit of 10 cells/ml, and was used as a rapid estimation of viable *Tetraselmis sp.* in the samples.

Bacteria

Generally the samples were diluted or concentrated to achieve a quantifiable concentration of colony forming units on a solid growth media (agar-medium plates) or a medium-amended filterpillow. The dilution or concentration was based on experience and expectance of the concentration of the bacteria in the sample. Dilution was performed by stepwise 10x dilution of the sample in a dilution series followed by incubation on agar. Concentration was performed by filtering a predetermined sample volume through a sterile filter followed by incubation of the filter on growth media.

Heterotrophic bacteria

Heterotrophic bacteria were quantified according to a modified version of Norwegian Standard NS-EN 6222:1999 using a marine agar for isolation of marine heterotrophic bacteria.

Coliforms

Coliform bacteria were quantified according to Norwegian Standard NS 4788 at a temperature of 37±1°C and an incubation period of 22-24 hours.

E.coli

E. coli were quantified according to Norwegian Standard NS 4792 or NS-EN ISO 9308-3 at a temperature of 44.5±0.2 °C and an incubation period of 18-24 hours.

Enterococcus group

Total fecal *Enterococci* were quantified according to Norwegian Standard NS-EN ISO 7899-2 at a temperature of 36±2 °C and an incubation period of 44 hours.

Intestinal *Enterococci*
Intestinal *Enterococci* were confirmed according to Norwegian Standard NS-EN ISO 7899-2 at a temperature of 44±0.2 °C and an incubation period of 2 hours.

Vibrio species *and Vibrio cholerae*
The total number of *Vibrio* sp., were determined by filtration of 1-100 ml sample, and by placing the filter on TCBS Cholera-medium agar plates (CMO333 from Oxoid). Plates were incubated at 37 °C, and colonies counted after 24 hours incubation. The TCBS Cholera-medium supports the growth of pathogenic Vibrios (e. g. *Vibrio cholerae*, *Vibrio parahaemolyticus*) as well as some other Vibrios and other bacterial species, i.e. *Aeromonas hydrophila.*

The strategy for elimination or identification of serotypes O1 and O139 were as follows:
The morphology of the colonies developing on the TCBS-medium after 24 h was visually studied. Colonies with distinct colour and morphology different from *Vibrio cholera* were not selected for further identification. Colonies with typical *Vibrio cholera* appearance were re-striked on TCBS medium and again inspected for growth and morphology. Classical elimination or identification methods were used, such as appearance in culture media and physiological and biochemical properties. If *Vibrio cholera* was identified, polymerase chain reaction (PCR) would be used for elimination or identification of the serotypes O1 and O139.

For samples with high number of *Vibrio spp.*, we use an optional method which is a modification of the method described by Huq *et al*. (2006). It is based on enrichment in alkaline peptone water (APW), followed by culturing of surface growth from APW on TCBS, and sub-culturing on nutrient agar without NaCl. It is a presence-absence method. The method should be performed for samples with high numbers of presumptive *Vibrio* spp. (>100 per 100 ml) where we have to confirm absence of *V. cholerae* in 100 ml water sample.

2.6.3 Toxicity measurements of treated ballast water

The following standard tests were performed on the treated ballast water.

- Growth inhibition of the marine alga *Skeletonema costatum* according to ISO 10253: Marine algal growth inhibition test.
- Acute toxicity to the marine crustacean *Acartia tonsa* according to ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*).
- Reproductive toxicity to the marine crustacean *Nitocra spinipes* according to "Forslag til Dansk Standard: Økotoksikologisk undersøgelse med krepsdyret *Nitocra spinipes*. Reproduksjonsmetode".
- The oyster embryo bioassay according to the ASTM method (E724) and the comprehensive guidelines laid out in the ecotoxicity test methods for effluent and receiving water assessment (EA, 2001).
- Acute toxicity towards the juvenile turbot (*Scopthalmus maximus*) according to OECD Guidelines for testing of chemicals (No. 203; Fish, acute toxicity test).
- Chronic toxicity towards the juvenile turbot (*Scopthalmus maximus*) according to OECD Guidelines for testing of chemicals" (No. 215; Fish, juvenile growth test), adapted for marine species.
- Chronic toxicity using rotatoria reproduction test with the marine species *Brachionus plicatilis* based on a standard test developed for the related freshwater species *Brachionus calyciflorus* (ISO 20666 – Determination of the chronic toxicity to *Brachionus calyciflorus*).

Sampling of treated ballast water for toxicity tests with algae and invertebrates
Test water for these tests was taken in connection with the general sampling routine of day 0 or day 5. All samples were grab samples transferred into 2 liter glass bottles (Sorvall). The bottles were

transported to the laboratory in cooling bags within 2 hours of sampling. Upon arrival at the laboratory the samples were put in a cooling room with a temperature of 4 °C. In general the test water was filtered through GF/C filter before being used in the tests. Further treatment of test water is described in the test reports (**Appendix K – Toxicity tests**).

Sampling of treated ballast water for toxicity tests with fish
Sampling of test water was undertaken directly after finishing the general sampling routine on day 5. A hose was connected to the gravity sampling hose and the test water was led directly to several 300 L stainless steel storage tanks in a climate room where the fish tests were undertaken. This allowed the water to acclimatise to the test temperature prior to use. Fish were exposed to the testwater without any other pre-treatment.

Growth inhibition of the marine alga *Skeletonema costatum*
The inhibitory effect of treated ballast water on the growth of the marine diatom *Skeletonema costatum*, strain NIVA BAC1, has been investigated. The test was performed according to ISO 10253: Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum.*

A concentration series of treated ballast water diluted in untreated water was prepared. The batches were inoculated with test algae and incubated on a shaking table at 20 ± 2 °C, under continuous illumination. Growth was monitored by daily counting of cell numbers using a Coulter Multisizer. The tests were performed with three replicates at each concentration and six control replicates in untreated ballast water.

The growth rate in each culture was calculated from the increase in cell density during three days exposure. Growth rates were calculated as percentage of growth rate in the controls (untreated ballast water) and plotted against concentration of treated ballast water. From the respons plot, the concentrations causing 10% and 50 % inhibition of the growth rate (i.e. EC_{10} and EC_{50}) were derived by non-linear regression analysis.

Reproductive toxicity to the marine crustacean *Nitocra spinipes*
The reproductive toxicity of treated ballast water to the marine crustacean *Nitocra spinipes* has been investigated. The test was performed according to Draft guideline for Danish Standard: ”Økotoksikologisk undersøgelse med krepsdyret *Nitocra spinipes*. Reproduksjonsmetode”. Test was performed as a limit test as defined in "OECD Guidelines for testing of chemicals" (No. 203; Fish, acute toxicity test), with one test concentration of 100 % treated ballast water and using non treated ballast water as control water.

The test was performed with 20 replicate vessels with 1 pregnant female in each vessel. The vessels were incubated for 14 days at 20 °C. The total number of living offspring was counted.

Acute toxicity to the marine crustacean *Acartia tonsa*
The acute toxicity of treated ballast water to the marine crustacean *Acartia tonsa* has been investigated. The test was performed according to ISO 14669: Determination of acute lethal toxicity to marine copepods (*Copepoda, Crustacea*). The test concentrations were in the range 32 to 100 % of treated ballast water.

The test was performed with four replicate vessels with 4-8 test animals for each test concentration and sixteen control replicate vessels. The vessels were incubated for 48 hours at 20 ± 1 °C. Mortality was recorded after 24 and 48 hours.

Chronic toxicity towards the juvenile turbot (*Scopthalmus maximus*)
The chronic toxicity of treated ballast water towards turbot was tested in accordance with the "OECD Guidelines for testing of chemicals" (No. 215; Fish, juvenile growth test), adapted for marine fish (McWilliams, 1994).

The testing of chronic toxicity of treated ballast water is required by the IMO G9 guidelines for testing of treatment technology. The fish was exposed continuously for 28 days with water exchange 3 times per

week. Test was performed as a limit test as defined in "OECD Guidelines for testing of chemicals" (No. 203; Fish, acute toxicity test), using one test concentration of 100 % treated ballast water and using non treated ballast water as control water.

The test water was taken directly from control and test tank (TT2 and TT1) (100% ballast water), after conditioning to the test temperature.

Acute toxicity towards the juvenile turbot (*Scopthalmus maximus*)
The acute toxicity of treated ballast water towards turbot was tested in accordance with the draft procedure of McWilliams (1994). The procedure follows the general guidelines of OECD 203 "Fish, Acute toxicity test".

The testing of acute toxicity of treated ballast water is required by the IMO G9 guidelines for testing of treatment technology. The fish was exposed continuously for 96 hours with full water exchange every day. Test was performed as a limit test as defined in "OECD Guidelines for testing of chemicals" (No. 203; Fish, acute toxicity test), using one test concentration of 100 % treated ballast water and using non treated ballast water as control water.

10 juvenile turbot were used in each aquarium with 40 l of medium. The test water was taken directly from the control and test tank (TT2 and TT1) (100% ballast water), after conditioning to test temperature.

The oyster embryo bioassay
The oyster embryo bioassay (OEB) is based on the ASTM method (E724) and the comprehensive guidelines laid out in the ecotoxicity test methods for effluent and receiving water assessment (EA, 2001). The OEB is a sensitive in vivo test that measures the response of the most sensitive life stage of the oyster to contaminant exposure. The bioassay measures the success of trocophore larvae to develop into a normal D-stage veliger larvae following 48 hour exposure to test media. The frequency of normal D-stage larvae following 48 hour exposure is determined microscopically to provide an assessment of sample toxicity. The data generated enables standard toxicity values such EC_{10}, EC_{50} and NOEC (no observable effect concentration) and LOEC (lowest observable effects concentration) values to be determined for the test sample.

Rotatoria reproduction test
The chronic toxicity to rotatoria was studied using the marine species *Brachionus plicatilis*. The rotifera were kept in a laboratory culture fed with live algae. The test procedure is based on a standard test developed for the related freshwater species *Brachionus calyciflorus* (ISO 20666 – Determination of the chronic toxicity to *Brachionus calyciflorus*). Briefly, freshly hatched rotatoria were incubated individually in a series of concentrations of the test water and in control water for 72 hours. At the end of the test, the number of egg and offspring were determined and compared with the control, i.e. the non treated ballast water. The population growth percentages were determined for each concentration of the test water.

3. Results and discussion

3.1 QA/QC procedures

Quality assurance and quality control have been performed during the testing according to Chapter 5 in the QAPP and according to G8. All activities and data collected during testing of the OceanGuard™ BWMS have been logged as summarized in **Table 5**. For each activity a specially designed log in paper and/or electronic format has been used (**Appendix A-K and P**). These log sheets were also used as quality assurance of some of the operations perfomed during the tests. For appendix L, M, N, O and Q, please refer to the QAPP made for this project.

Table 5. Log protocols for all activities in the project.

Appendix	Description
A	Total project management
B	Chemical water quality preparation/Homogeneity
C	Biological water quality preparation
D	Operational data
E	Gas measurements
F	WST check list before each test
G	Logging of *in situ* measurements
H	Evaluation form for organisms ≥50 µm
I	Evaluation form for organisms ≥10-50 µm
J	Evaluation form for heterotrophic bacteria, coliforms, *E. coli*, Enterococcus group, intestinal *Enterococci*, *Vibrio cholerae* and *Vibrio cholerae* (serotypes O1 and O139).
K	Toxicity tests
L	Process description
M	System start-up procedures
N	Laboratory sub-contractors
O	Disinfection by-products methods and detection limits
P	Measurements of free and total chlorine (TRO)
Q	Material data sheet

3.2 Operational performance of the QHT BMWS

A total of 13 test cycles have been completed in the period of 22nd July 2009 to 26th October 2009. Test cycles 1, 2, 3, 4, 5, 11, 12 and 13 were conducted with medium salinity water (brackishwater < 22 PSU) and the remaining test cycles (6, 7, 8, 9 and 10) with high salinity (seawater >32 PSU).

Each test cycle lasted for 5 days. The dates of the cycles and the time for start/stop of each step in the test cycle are given in **Table 6**.

A control of the operational performance of the technology was performed in each test cycle by the site responsible person from NIVA. Reports from these controls are included in **Appendix D**. Check points and operational parameters for documenting the performance of the treatment technology were included and monitored in this quality control, including the time for start and stop of ballasting and deballasting, and start and stop of ballasting and deballasting of control water.

Flow rate were recorded every fifth minutes during the tests cycles. Flow meter was placed inside the QHT container and was read by the QHT personell or NIVA personell, and immediately communicated to NIVAs site responsible. The averages of multiple flow measurements are given in

Table 7. Flowrates were also calculated from the time for start/stop and the volume of the tanks, which give an accurate flow estimate. The ranges of calculated flowrates for all test cycles are given in the lower row of **Table 7**. As observed, the measured flow rates are in general in the mid to higher of the the calculated ranges, indicating that the flowmeter is relatively consistent with the actual calculated flow.

It was observed a technical failure in auxiliary equipment (air compressor, supplied by NIVA, for filter operation) during test cycle 2, which may have influenced the treatment efficiency and the biological data. This cycle should not be included in the biological efficiency evaluation of the BWMS, with exception of the toxicity data retrieved from this cycle, as the concentration of active substance was not influenced by the technical failure.

Table 6. Test cycles completed with dates and the time for start/stop of each step in the cycle.

Test cycle	Date	Start/stop ballasting WST to TT2 Day 0	Start/stop deballasting TT2 to TT1 Day 5	Start/stop control ballasting WST to CT2 Day 0	Start/stop control deballasting CT2 to TT2 Day 5	Salinity PSU
1	22nd-27th july 2009	60 min	45 min	50 min	43 min	<22
2*	29th july- 03rd august 2009	58 min	48 min	40min	38 min	<22
3	5th - 10th august 2009	55 min	46 min	43 min	41 min	<22
4	12th -17th august 2009	52 min	42 min	44 min	41 min	<22
5	19th - 24th august 2009	60 min	44 min	42 min	40 min	<22
6	01st - 06th September 2009	42 min	37 min	39 min	39 min	>32
7	09th - 14th september 2009	43 min	40 min	38 min	37 min	>32
8	16th - 21th september 2009	41 min	39 min	40 min	39 min	>32
9	23th - 28th september 2009	42 min	38 min	40 min	39 min	>32
10	30th sept.- 05th october 2009	43 min	36 min	41 min	40 min	>32
11	07th - 12th october 2009	46 min	39 min	41 min	39 mim	<22
12	14th - 19th october 2009	47 min	39 min	41 min	40 min	<22
13	21th - 26th october 2009	45 min	43 min	41 min	40 min	<22

*Technical failure

Table 7. Average flowrates based on measurements and calculations (lower row).

Test cycle	Dates	Average flow ballasting m^3/h	Average flow deballasting m^3/h	Average flow control ballasting m^3/h	Average flow control deballasting m^3/h
1	22nd-27th july 2009	269	315	290	315
2	29th july- 03rd august 2009	308	308	308	318
3	5th - 10th august 2009	297	302	346	310
4	12th -17th august 2009	278	310	310	317
5	19th - 24th august 2009	272	306	317	315
6	01st - 06th September 2009	340	346	346	344
7	09th - 14th september 2009	328	319	345	343
8	16th - 21th september 2009	333	339	337	342
9	23th - 28th september 2009	337	339	357	339
10	30th sept.- 05th october 09	327	340	327	332
11	07th - 12th october 2009	310	331	333	340
12	14th - 19th october 2009	314	335	341	341
13	21th - 26th october 2009	308	312	342	346
Flow range for all test cycles based on flowmeter measurements		239-354	160-443	259-389	298-400
Average flow range for all test cycles based on flowmeter measurements		269-340	302-346	290-357	310-346
Average flow range for all test cycles based on calculations		227-333	276-326	254-351	276-339

3.3 Chemical water quality criteria

Prior to sampling, the homogeneity of the water in the tanks were ensured by measuring the turbidity at several locations in the tanks. No sampling was undertaken before the variation in turbidity in the tank was less then 10%. All results from turbidity measurements are given in **Table 8** and shows that variations of the turbidity in each tank were always less than 10% during sampling.

The general lower turbidity in control water than in treated water on day 5 can be explained by co-precipitation and "sweep" settling of larger and finer particles present in the control tank. These mechanisms may enhance the sedimentation of finer particles which contribute more than the larger particles to high turbidity values.

Table 8. Average turbidity measurements (NTU/FNU) with standard deviation (stdev) in ballast tanks.

Test nr	Tank	Day 0			Day 5		
		Average	Stdev	% stdev	Average	Stdev	% stdev
Test 1	Influent	32.4	0.7	2.2	-	-	-
	Treated	19.0	0.6	3.1	11.4	0.1	0.9
	Control	14.0	0.2	1.7	4.1	0.2	4.9
Test 2	Influent	34.0	1.0	2.9	-	-	-
	Treated	32.1	0.8	2.4	17.3	0.2	1.2
	Control	28.5	0.7	2.4	8.0	0.2	2.8
Test 3	Influent	42.2	0.8	1.8	-	-	-
	Treated	43.1	1.8	4.3	18.7	0.3	1.6
	Control	40.0	1.9	4.7	8.9	0.2	1.7
Test 4	Influent	24.1	0.1	0.5	-	-	-
	Treated	23.6	0.7	2.9	10.0	0.0	0.0
	Control	19.9	0.7	3.7	9.8	0.2	1.7
Test 5	Influent	42.8	0.3	0.8	-	-	-
	Treated	43.9	0.5	1.1	14.4	0.1	0.9
	Control	42.7	0.9	2.0	10.6	0.2	1.9
Test 6	Influent	2.9	0.2	5.3	-	-	-
	Treated	2.9	0.1	4.0	2.6	0.2	6.1
	Control	2.9	0.2	5.7	0.8	0.1	7.3
Test 7	Influent	3.5	0.3	7.9	-	-	-
	Treated	2.8	0.1	4.9	2.0	0.1	6.9
	Control	2.8	0.0	1.5	0.6	0.1	9.1
Test 8	Influent	3.7	0.1	1.7	-	-	-
	Treated	2.9	0.2	6.7	2.4	0.1	0.0
	Control	3.6	0.3	7.5	1.4	0.1	8.3
Test 9	Influent	3.5	0.2	6.5	-	-	-
	Treated	3.2	0.2	7.2	2.3	0.2	7.0
	Control	3.5	0.1	3.1	1.5	0.1	3.5
Test 10	Influent	3.5	0.2	5.4	-	-	-
	Treated	3.5	0.1	3.3	2.9	0.1	2.6
	Control	3.3	0.1	1.7	2.1	0.2	7.7
Test 11	Influent	42.3	1.3	3.0	-	-	-
	Treated	41.5	0.9	2.1	29.0	0.9	3.2
	Control	41.5	1.1	2.6	17.1	0.3	1.9
Test 12	Influent	45.8	0.4	3.0	-	-	-
	Treated	41.5	0.9	2.1	25.8	0.7	2.7
	Control	41.5	1.1	2.6	13.1	0.2	1.6
Test 13	Influent	35.8	0.5	1.4	-	-	-
	Treated	36.9	0.4	1.0	20.1	1.0	5.0
	Control	35.7	0.8	2.1	10.9	0.2	2.1

The temperature, salinity, pH, dissolved oxygen content, total residual oxidants (TRO) as free and total chlorine, and redox were measured in the test water (WST) prior to treatment, upon completion of ballasting and at day 1, 2 and 5 (before and after deballasting) in the storage tanks TT2 and CT2 in each test cycle. The results are given in **Table 9** and shows that the water quality parameters are relatively stable during each test cycle. The results for salinity show that the requirement for the two testwater qualities to be separated by at least 10 PSU is fulfilled.

Table 9. Temperature, salinity, pH, redox, dissolved oxygen and TRO as free and total chlorine in the test water tank (WST) prior to treatment and in the storage tanks TT1 (treated water) and CT2 (control water) for each test cycle. (b.d = before deballasting. a.d = after deballasting).

		WST	Treated water							Control water						
	Day	0	0	1	2	3	4	5 (b.d)	5 (a.d)	0	1	2	3	4	5 (b.d.)	5 (a.d)
Parameter	Unit															
Test cycle 1																
Temperature	ºC	18.60	18.80	18.50	18.70	-	-	18.20	18.20	18.80	18.50	18.60	-	-	18.20	18.20
pH	-	8.18	8.11	8.07	7.80	-	-	7.78	7.93	8.24	8.20	7.99	-	-	7.85	7.91
Dissolved O_2	mg/l	8.0	7.9	7.5	7.9	-	-	7.8	6.4	7.0	7.0	7.2	-	-	7.1	5.8
Salinity	PSU	21.2	21.2	21.2	21.2	-	-	21.2	21.2	21.2	21.2	21.2	-	-	21.2	21.2
Redox	mV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free chlorine stdev	mg/l	0.03 ±0.01	0.73 ±0.03	0.02 ±0.00	<0.02	-	-	<0.02	<0.02	<0.02	<0.02	0.02 ±0.02	-	-	<0.02	<0.02
Total chlorine stdev	mg/l	0.04 ±0.00	0.95 ±0.04	0.04 ±0.01	0.02 ±0.01	-	-	<0.02	<0.02	0.02 ±0.01	<0.02	<0.02	-	-	<0.02	<0.02
Test cycle 2																
Temperature	ºC	18.30	18.40	18.40	18.10	-	-	18	17.9	18.60	18.40	18.00	-	-	17.8	17.80
pH	-	8.20	8.14	8.10	8.08	-	-	7.68	7.8	8.29	8.25	8.19	-	-	7.91	7.97
Dissolved O_2	mg/l	8.5	7.9	7.2	6.9	-	-	6.2	6.3	8.3	8.0	7.5	-	-	6.4	6.6
Salinity	PSU	21.4	21.3	21.3	21.3	-	-	21.3	21.3	21.3	21.3	21.3	-	-	21.3	21.3
Redox	mV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Free chlorine stdev	mg/l	<0.02	0.32 ±0.02	<0.02	<0.02	-	-	0.02	<0.02	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine stdev	mg/l	0.02 ±0.01	0.40 ±0.01	<0.02	<0.02	-	-	<0.02	<0.02	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Test cycle 3																
Temperature	ºC	17.10	17.30	17.30	17.50	-	-	18	17.90	17.40	17.40	17.70	-	-	18	18.10
pH	-	8.02	7.33	7.06	7.04	-	-	7.6	7.74	7.31	7.27	7.26	-	-	7.71	7.62
Dissolved O_2	mg/l	8.3	8.3	8.2	8.3	-	-	6.6	6.6	8.3	8.0	7.6	-	-	6.1	6.3
Salinity	PSU	21.5	21.6	21.6	-	-	-	21.7	21.7	21.6	21.7	-	-	-	21.7	21.8
Redox	mV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free chlorine stdev	mg/l	<0.02	0.45 ±0.02	<0.02	<0.02	-	-	0.02	0.02 ±0.01	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine stdev	mg/l	<0.02	0.62 ±0.03	0.02 ±0.01	<0.02	-	-	<0.02	<0.02	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Test cycle 4																
Temperature	ºC	17.50	17.60	17.50	17.20	-	-	16.8	16.90	17.60	17.60	17.20	-	-	16.9	17.10
pH	-	8.16	7.95	7.98	7.90	-	-	7.93	7.97	7.42	7.54	8.04	-	-	8.05	8.05
Dissolved O_2	mg/l	10.0	9.7	8.4	9.4	-	-	7	7.8	9.7	8.8	8.6	-	-	7.1	6.1
Salinity	PSU	21.8	21.8	21.8	21.8	-	-	21.8	21.8	21.8	21.8	21.8	-	-	21.7	21.8
Redox	mV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free chlorine stdev	mg/l	0.02 0.01	0.51 0.02	0.03 0.01	<0.02	-	-	0.02	<0.02	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine stdev	mg/l	<0.02	0.69 ±0.11	0.04 ±0.01	0.02 ±0.01	-	-	<0.02	<0.02	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Test cycle 5																
Temperature	ºC	14.60	14.70	14.90	15.50	-	-	15.6	15.5	14.80	14.90	15.50	-	-	15.5	15.70
pH	-	8.14	8.00	7.92	7.90	-	-	7.41	7.74	8.17	8.14	8.07	-	-	7.86	7.85
Dissolved O_2	mg/l	8.4	8.1	7.8	7.1	-	-	7.1	8.4	8.4	8.1	7.5	-	-	7.2	7.4

Salinity	PSU	22.6	22.6	22.6	22.6	-	-	22.6	22.6	22.6	22.6	22.7	-	-	22.6	22.6
Redox	mV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free chlorine stdev	mg/l	<0.02	1.86 ±0.06	0.04 ±0.01	0.02 ±0.01	-	-	<0.02	0.02 ±0.01	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine stdev	mg/l	0.02 ±0.01	1.93 ±0.03	0.15 ±0.02	0.05 ±0.02	-	-	<0.02	0.02 ±0.01	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Test Cycle 6																
Temperature	ºC	11.40	11.60	11.60	12.00	-	-	12.1	12.20	11.50	11.70	12.10	-	-	12.2	12.30
pH	-	8.03	8.01	7.96	7.95	-	-	8.08	8.05	8.05	8.04	8.00	-	-	8.01	8.01
Dissolved O_2	mg/l	8.5	8.2	8.1	7.7	-	-	8.1	8.1	8.3	8.0	7.9	-	-	7.2	7.2
Salinity	PSU	32.8	32.6	32.7	32.7	-	-	32.6	32.6	32.6	32.6	32.6	-	-	32.5	32.5
Redox	mV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free chlorine stdev	mg/l	<0.02	1.22 ±0.01	0.19 ±0.01	0.07 ±0.01	-	-	0.02 ±0.01	0.02 ±0.01	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine stdev	mg/l	<0.02	1.28 ±0.02	0.31 ±0.02	0.16 ±0.01	-	-	0.03 ±0.01	0.04 ±0.00	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Test Cycle 7																
Temperature	ºC	11.30	11.40	11.80	11.80	-	-	12.1	12.30	11.50	11.80	11.90	-	-	12.20	12.30
pH	-	8.16	8.08	8.00	8.02	-	-	7.94	7.92	8.14	8.14	8.12	-	-	7.96	7.95
Dissolved O_2	mg/l	8.4	8.1	8.1	7.8	-	-	7.9	8.3	8.3	8.3	8.2	-	-	7.2	7.6
Salinity	PSU	32.4	32.4	32.4	32.4	-	-	32.4	32.4	32.4	32.4	32.4	-	-	32.4	32.4
Redox	mV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free chlorine stdev	mg/l	<0.02	1.33 ±0.04	0.36 ±0.02	0.19 ±0.01	-	-	0.02 ±0.01	0.03 ±0.01	0.02 ±0.01	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine stdev	mg/l	0.02 ±0.01	1.39 ±0.04	0.44 ±0.03	0.31 ±0.01	-	-	0.09 ±0.01	0.09 ±0.01	0.03 ±0.00	0.02 ±0.01	0.02 ±0.01	-	-	<0.02	<0.02
Test Cycle 8																
Temperature	ºC	10.20	10.60	10.60	10.70	-	-	10.60	11.20	10.60	10.60	10.70	-	-	10.6	11.10

pH	-	8.04	7.93	7.99	7.97	-	-	7.97	7.97	8.07	8.07	8.04	-	-	8	8.00
Dissolved O_2	mg/l	8.7	8.3	8.7	8.8	-	-	7.8	7.8	8.5	8.6	8.8	-	-	8.2	8.2
Salinity	PSU	32.0	31.8	32.0	32.0	-	-	32.0	32.0	32.0	32.0	32.0	-	-	32	32.0
Redox	mV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free chlorine stdev	mg/l	<0.02	1.49 ±0.02	0.36 ±0.02	0.18 ±0.01	-	-	0.04 ±0.02	0.03 ±0.01	<0.02	<0.02	<0.02	-	-	0.02 ±0.01	<0.02
Total chlorine stdev	mg/l	<0.02	1.46 ±0.03	0.51 ±0.05	0.31 ±0.01	-	-	0.11 ±0.01	0.09 ±0.01	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Test Cycle 9																
Temperature	ºC	9.40	9.50	9.70	9.80	-	-	10.2	10.30	9.60	9.70	9.90	-	-	10.2	10.40
pH	-	8.04	7.95	7.94	7.94	-	-	7.93	7.91	8.05	8.05	8.03	-	-	7.97	7.95
Dissolved O_2	mg/l	9.0	8.8	8.5	8.1	-	-	7.8	8.0	9.0	8.7	8.6	-	-	8.1	8.2
Salinity	PSU	32.2	32.2	32.2	32.2	-	-	32.2	32.2	32.2	32.2	32.2	-	-	32.2	32.2
Redox	mV	254	675	587	402	-	-	301	329	371	379	370	-	-	273	291
Free chlorine stdev	mg/l	<0.02	1.29 ±0.02	0.21 ±0.01	0.10 ±0.01	-	-	0.03 ±0.01	0.02 ±0.01	<0.02	<0.02	<0.02	-	-	<0.02	0.02 ±0.01
Total chlorine stdev	mg/l	<0.02	1.23 ±0.08	0.29 ±0.01	0.16 ±0.01	-	-	0.05 ±0.01	0.05 ±0.01	0.02 ±0.01	0.02 ±0.01	<0.02	-	-	<0.02	<0.02
Test Cycle 10																
Temperature	ºC	8.40	8.50	8.40	8.40	-	-	7.8	7.70	8.50	8.40	8.30	-	-	7.7	7.70
pH	-	8.00	7.91	7.90	7.90	-	-	7.94	7.99	8.00	8.00	8.00	-	-	7.98	8.00
Dissolved O_2	mg/l	9.0	8.7	8.6	8.6	-	-	8.3	7.9	8.6	8.6	8.5	-	-	8	8.0
Salinity	PSU	32.2	32.2	32.2	32.2	-	-	32.2	32.2	32.2	32.2	32.2	-	-	32.2	32.2
Redox	mV	382	724	673	532	-	-	401	370	389	407	400	-	-	390	299
Free chlorine stdev	mg/l	0.02 ± 0.01	1.27 ± 0.12	0.34 ± 0.02	0.18 ± 0.01	-	-	0.04 ± 0.01	0.04 ± 0.01	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine	mg/l	<0.02	1.16	0.43	0.25	-	-	0.07	0.08	0.02	0.02	0.02	-	-	0.02	0.02

stdev			± 0.07	± 0.02	± 0.02			± 0.01	± 0.01	± 0.01	± 0.01	± 0.01			± 0.01	±0.01
Test Cycle 11																
Temperature	ºC	10.40	10.40	10.10	9.80	-	-	8.7	8.70	10.40	10.10	9.80	-	-	8.6	8.70
pH	-	8.08	7.91	7.90	7.87	-	-	7.83	7.85	8.08	8.07	8.00	-	-	7.8	7.82
Dissolved O_2	mg/l	8.4	7.8	8.1	8.2	-	-	7.9	8.0	8.2	8.2	8.0	-	-	7.6	7.7
Salinity	PSU	22.0	22.0	22.0	22.0	-	-	22.0	22.0	22.0	22.0	22.0	-	-	22.0	22.0
Redox	mV	315	736	389	305	-	-	277	241	323	331	274	-	-	292	279
Free chlorine stdev	mg/l	<0.02	1.98 ± 0.02	0.06 ± 0.01	0.03 ± 0.01	-	-	0.02 ± 0.00	0.02 ± 0.02	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine stdev	mg/l	<0.02	2.37 ± 0.05	0.16 ± 0.02	0.07 ± 0.01	-	-	0.03 ± 0.01	0.02 ± 0.01	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Test Cycle 12																
Temperature	ºC	7.70	7.70	7.20	6.90	-	-	6.3	6.30	7.70	7.30	7.00	-	-	6.3	6.20
pH	-	8.01	7.88	7.83	7.84	-	-	7.55	7.56	8.00	8.00	8.0	-	-	7.58	7.78
Dissolved O_2	mg/l	8.6	8.3	8.2	8.0	-	-	9.8	10.1	8.4	8.0	7.9	-	-	8.6	8.9
Salinity	PSU	21.6	21.6	21.6	21.6	-	-	21.5	21.5	21.6	21.6	21.6	-	-	21.5	21.5
Redox	mV	280.6	737.1	425.3	279.7	-	-	244.0	-	292.2	266.2	263.7	-	-	359.0	363.0
Free chlorine stdev	mg/l	-	2.29 ± 0.30	0.36 ± 0.04	0.14 ± 0.03	-	-	0.06 ± 0.02	0.05 ± 0.03	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Total chlorine stdev	mg/l	-	2.74 ± 0.11	0.43 ± 0.03	0.19 ± 0.05	-	-	0.08 ± 0.01	0.07 ± 0.01	<0.02	<0.02	<0.02	-	-	<0.02	<0.02
Test Cycle 13																
Temperature	ºC	8.80	8.80	8.70	8.40	-	-	7.9	7.90	8.70	8.50	8.30	-	-	7.9	7.90
pH	-	7.76	7.82	7.61	7.56	-	-	7.62	7.64	7.81	7.77	7.7	-	-	7.66	7.92
Dissolved O_2	mg/l	8.1	8.2	8.3	8.0	-	-	7.8	7.7	8.1	8.0	7.7	-	-	6.8	7.1

Salinity	PSU	21.5	21.5	21.5	21.5	-	-	21.5	21.5	21.5	21.6	21.6	-	-	21.5	21.5
Redox	mV	357.0	602	439.0	213.0	-	-	226.0	191.0	362.0	280.0	359.0	-	-	255.0	202.0
Free chlorine stdev	mg/l	<0.02	1.75 ± 0.00	0.23 ± 0.03	0.09 ± 0.01	-	-	0.04 ± 0.03	0.02 ± 0.01	<0.02	<0.02	<0.02	-	-	<0.02	0.02 ±0.02
Total chlorine stdev	mg/l	<0.02	1.85 ± 0.21	0.28 ± 0.01	0.14 ± 0.01	-	-	0.09 ± 0.05	0.04 ± 0.01	<0.02	<0.02	<0.02	-	-	0.02 ± 0.02	<0.02

3.3.1 Chemical water quality of test water

Concentration of total suspended solids (TSS), dissolved organic carbon (DOC) and particulate organic carbon (POC) at day 0 in WST, TT2 and CT2, and at day 5 in TT2 and TT1 in the different test cycles are shown in **Table 10**. The chemical water quality requirements were fulfilled for all tests.

Table 10. Chemical water quality (average and standard deviation of triplicate samples). Green background indicates that required level was fulfilled.

TEST 1	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ =30 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level. influent water	>50	-	>5	-	>5	-
Influent water (WST)	50.0	2.7	7.3	0.1	6.8	0.3
Treated day 0 (TT2)	40.8	1.7	7.3	0.1	5.0	0.4
Treated day 5 (TT1)	11.7	1.0	6.5	0.1	1.4	0.0
Control day 0 (CT2)	35.1	0.8	7.2	0.1	5.0	0.7
Control day 5 (TT2)	19.9	1.0	6.2	0.1	1.4	0.0
TEST 2	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ =30 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level. influent water	>50	-	>5	-	>5	-
Influent water (WST)	65.6	2.5	6.7	0.2	7.8	0.6
Treated day 0 (TT2)	62.2	0.6	6.6	0.2	6.9	0.7
Treated day 5 (TT1)	16.7	2.0	5.9	0.1	1.3	0.1
Control day 0 (CT2)	58.0	1.3	6.4	0.0	6.2	0.2
Control day 5 (TT2)	28.3	2.8	5.8	0.2	1.1	0.0
TEST 3	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ =30 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level. influent water	>50	-	>5	-	>5	-
Influent water (WST)	80.2	2.8	6.6	0.2	7.8	0.8
Treated day 0 (TT2)	80.9	3.3	6.6	0.1	7.5	0.7
Treated day 5 (TT1)	6.0	-	5.1	-	0.3	-
Control day 0 (CT2)	75.3	5.2	6.4	0.1	7.5	1.1
Control day 5 (TT2)	13.7	-	4.9	-	0.4	-

TEST 4	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ =30 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level. influent water	>50	-	>5	-	>5	-
Influent water (WST)	50.0	0.6	7.0	0.2	7.8	0.6
Treated day 0 (TT2)	47.1	5,1	7.3	0.0	6.6	0.3
Treated day 5 (TT1)	21.9	0.8	6.1	0.1	2.8	0.1
Control day 0 (CT2)	42.9	2.6	6.9	0.1	7.1	0.4
Control day 5 (TT2)	20.9	4.7	6.5	0.9	1.9	0.7
TEST 5	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ =30 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level. influent water	>50	-	>5	-	>5	-
Influent water (WST)	83.5	1.8	6.6	0.2	8.5	0.3
Treated day 0 (TT2)	83.9	0.7	6.5	0.1	7.7	0.5
Treated day 5 (TT1)	16.2	0.5	5.8	0.1	1.3	0.0
Control day 0 (CT2)	81.1	0.9	6.5	0.1	8.3	0.4
Control day 5 (TT2)	16.2	0.5	5.8	0.1	1.3	0.0
TEST 6	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level. influent water	>1	-	>1	-	>1	-
Influent water (WST)	12.6	5.1	2.6	0.2	2.6	0.1
Treated day 0 (TT2)	11.0	0.0	2.6	0.1	2.2	0.1
Treated day 5 (TT1)	4.2	0.6	2.3	0.1	0.6	0.1
Control day 0 (CT2)	12.3	1.2	2.8	0.3	2.6	0.0
Control day 5 (TT2)	6.7	0.4	2.7	0.1	1.1	0.0
TEST 7	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level. influent water	>1	-	>1	-	>1	-
Influent water (WST)	13.8	2.2	2.6	0.1	2.5	0.2

Treated day 0 (TT2)	11.4	0.3	2.7	0.1	2.3	0.0
Treated day 5 (TT1)	6.3	0.3	2.3	0.1	0.6	0.0
Control day 0 (CT2)	12.1	0.3	2.5	0.1	2.3	0.1
Control day 5 (TT2)	8.2	0.6	2.7	0.1	1.0	0.0
TEST 8	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level. influent water	>1	-	>1	-	>1	-
Influent water (WST)	14.3	3.8	2.6	0.1	2.5	0.1
Treated day 0 (TT2)	11.8	1.0	2.7	0.2	2.2	0.1
Treated day 5 (TT1)	5.8	2.5	2.4	0.1	0.8	0.3
Control day 0 (CT2)	12.2	0.2	2.7	0.3	2.5	0.1
Control day 5 (TT2)	5.5	1.3	2.5	0.1	0.7	0.2
TEST 9	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level. influent water	>1	-	>1	-	>1	-
Influent water (WST)	16.3	4.2	2.5	0.1	2.3	0.1
Treated day 0 (TT2)	11.8	2.4	2.5	0.1	2.2	0.1
Treated day 5 (TT1)	7.2	1.3	2.9	0.1	0.7	0.0
Control day 0 (CT2)	14.0	0.6	2.5	0.0	2.4	0.2
Control day 5 (TT2)	5.5	1.0	2.4	0.3	1.1	0.1
TEST 10	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ = 2.0 kg**		**$DOC_{Additive}$ = 2.5 kg**		**$POC_{Additive}$ = 3.0 kg**	
Required level. influent water	>1	-	>1	-	>1	-
Influent water (WST)	11.6	0.6	2.4	0.2	2.6	0.1
Treated day 0 (TT2)	11.9	1.1	2.3	0.1	2.2	0.1
Treated day 5 (TT1)	8.6	1.3	2.2	0.1	0.8	0.1
Control day 0 (CT2)	11.8	0.6	2.3	0.1	2.3	0.0
Control day 5 (TT2)	11.8	0.6	2.0	0.1	1.4	0.0

TEST 11	TSS mg/l		DOC mg/l		POC mg/l	
	Average	Stdev	Average	Stdev	Average	Stdev
Additions to influent water	**$TSS_{Additive}$ =30 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level. influent water	>50	-	>5	-	>5	-
Influent water (WST)	85.1	1.3	6.2	0.2	8.1	0.1
Treated day 0 (TT2)	82.2	0.8	6.2	0.1	7.8	0.3
Treated day 5 (TT1)	33.0	4.8	5.2	0.1	2.5	0.1
Control day 0 (CT2)	84.0	0.0	6.1	0.0	7.6	0.9
Control day 5 (TT2)	49.8	1.6	6.1	0.3	3.0	0.1
TEST 12	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ =30 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level. influent water	>50	-	>5	-	>5	-
Influent water (WST)	85.9	3.2	5.6	0.2	7.5	1.3
Treated day 0 (TT2)	85.1	1.7	5.3	0.1	8.2	0.1
Treated day 5 (TT1)	27.4	1.9	5.0	0.1	1.8	0.2
Control day 0 (CT2)	81.3	1.2	5.4	0.1	7.4	0.2
Control day 5 (TT2)	42.9	2.5	5.7	0.4	3.0	0.3
TEST 13	**TSS mg/l**		**DOC mg/l**		**POC mg/l**	
	Average	**Stdev**	**Average**	**Stdev**	**Average**	**Stdev**
Additions to influent water	**$TSS_{Additive}$ =30 kg**		**$DOC_{Additive}$ = 10 kg**		**$POC_{Additive}$ = 12.5 kg**	
Required level. influent water	>50	-	>5	-	>5	-
Influent water (WST)	79.7	1.5	5.6	0.2	7.6	0.5
Treated day 0 (TT2)	79.0	2.6	5.5	0.1	7.3	0.3
Treated day 5 (TT1)	20.9	1.3	4.7	0.1	1.5	0.1
Control day 0 (CT2)	77.0	4.3	5.4	0.1	6.9	0.6
Control day 5 (TT2)	42.7	3.0	5.7	0.3	2.3	0.1

3.3.2 Disinfection by-products

A range of disinfection by products were analysed in treated water and control water on day 0 and after five days storage (day 5). Disinfection by products found in concentrations above the detection limit in treated water are shown in **Table 11** as average of seven brackish water test cycles, test 1-5 and test 12-13 (only day 5 samples from test 12-13), and five seawater tests (test 6-10). The ranges of DBP concentration are included to demonstrate the variation of each compound between parallel tests. A summary of all DBP-results are given in Appendix 1.

Table 11. Average and range values of dominating disinfection by-products detected in seven brackish water test cycles, test 1-5 and test 12-13 (only day 5 samples from test 12-13) and five seawater test cycles (test 6-10).

Compound	Unit	Brackish water		Seawater	
		Day 0	Day 5	Day 0	Day 5
Trichloromethane (chloroform)	µg/l	0.03 <0.1-0.1	0.04 <0.1-0.1	<0.10	0.03 <0.10-0.10
Bromodichloromethane	µg/l	0.33 0.2-0.4	0.5 0.3-0.8	<0.10	0.3 0.2-0.4
Dibromochloromethane	µg/l	9.47 7.4-11.0	12.8 9.2-18.0	1.33 1.0-1.7	4.9 4.4-5.7
Tribromomethane (bromoform)	µg/l	233 190-280	416 290-670	39 26-52	140 120-170
Dibromoacetic acid (DBAA)	µg/l	8.9 5.1-12.0	1.8 <0.1-3.1	9.8 0.95-26	3.7 1.1-8.5
Dichlorobromoacetic acid (DCBAA)	µg/l	<0.1	0.03 <0.1-0.13	<0.1	<0.1
Bromochloroacetic acid (BCAA)	µg/l	0.3 0.1-0.4	0.03 <0.1-0.3	0.1 <0.1-0.31	0.04 <0.1-0.11
2,4,6-Tribromophenol	µg/l	0.03 <0.1-0.1	<0.1	0.03 <0.10-0.10	0.03 <0.10-0.10
Monobromoacetonitrile	µg/l	0.2 <0.1-0.6	<0.1	<0.10	<0.10
Dibromoacetonitrile	µg/l	<0.1	<0.1	0.2 <0.1-0.5	0.3 <0.1-0.8
Dibromomethane	µg/l	0.07 <0.1-0.2	0.4 <0.1-1.3	0.2 <0.10-0.3	0.3 <0.1-0.4
AOX	mg/l	0.11 0.08-0.13	0.16 0.10-0.32	0.02 0.01-0.02	0.07 0.06-0.09
EOX	mg/l	0.02 0.02-0.02	0.03 0.02-0.05	<0.010	0.02 0.02-0.02
Bromate	µg/l	6.2 5.5-7.2	3.4 4.4-6.6	2.3 1.9-2.6	1.2 1.7-1.9

Chemical fate analysis of disinfection by-products

During the five days storage period and after deballasting on day 5, a study of the concentration of selected disinfection by-products in treated water was done. The study was performed for two test cycles, test cycle 5 (brackish water) and test cycle 9 (seawater). For Cycle 5, analyses were only done for by-products that were detected in the previous test cycles, but for Cycle 10, all DBPs were analysed. Measurements were done on day 0, 2 and 5 after treatment (ballasting), and during a 48h period starting immediately after second treatment (deballasting). **Figure 2, 3, 4** and 5 shows the result from compounds that were detected in the treated water in test cycle 5 (brackish water) and test cycle 9 (seawater). An overview of all the results obtained from treated water and control water are shown in Appendix 2 – Chemical fate study of disinfection by-products, test cycle 5 and test cycle 9.

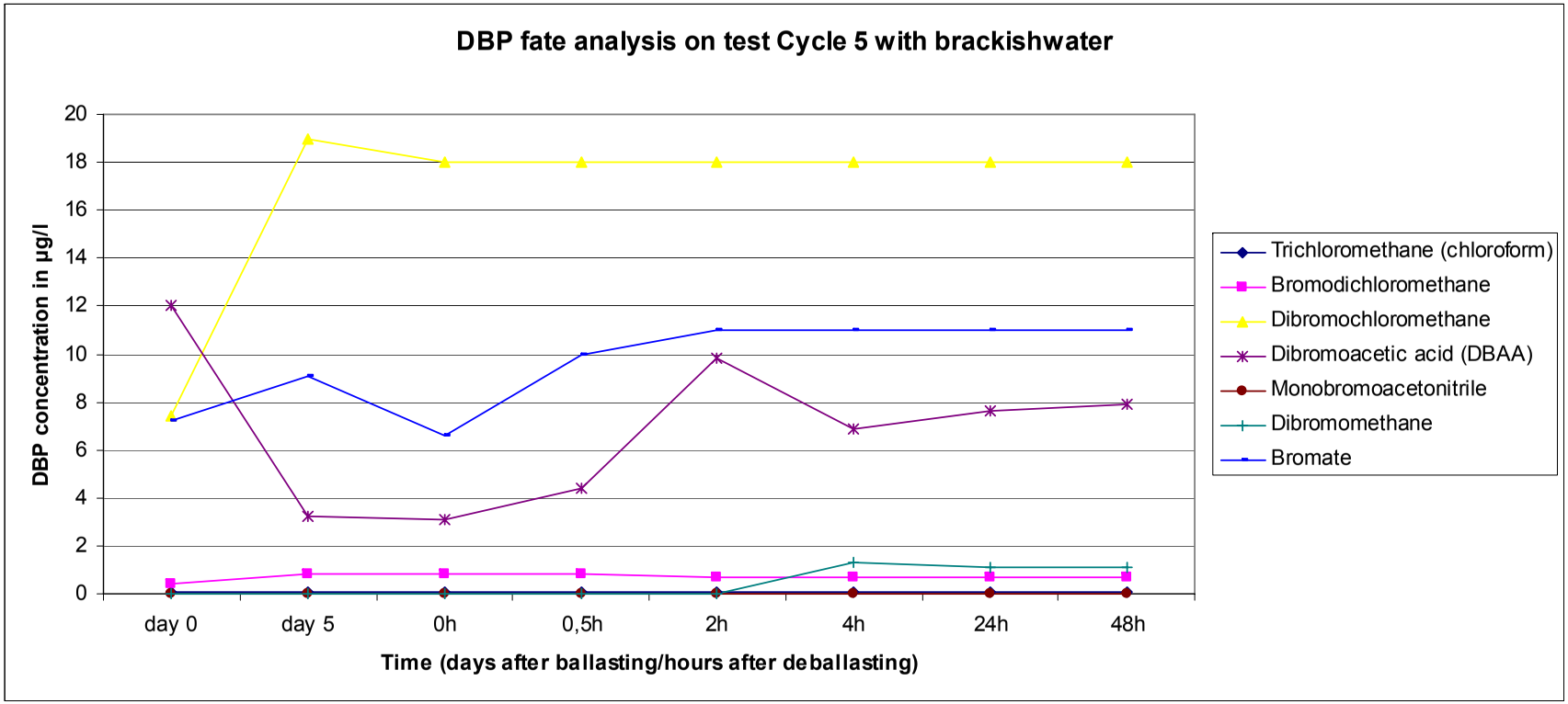

Figure 2 Concentration of disinfection by-products (µg/l) in treated brackish water in test cycle 5, measured at defined days after ballasting and defined hours after deballasting.

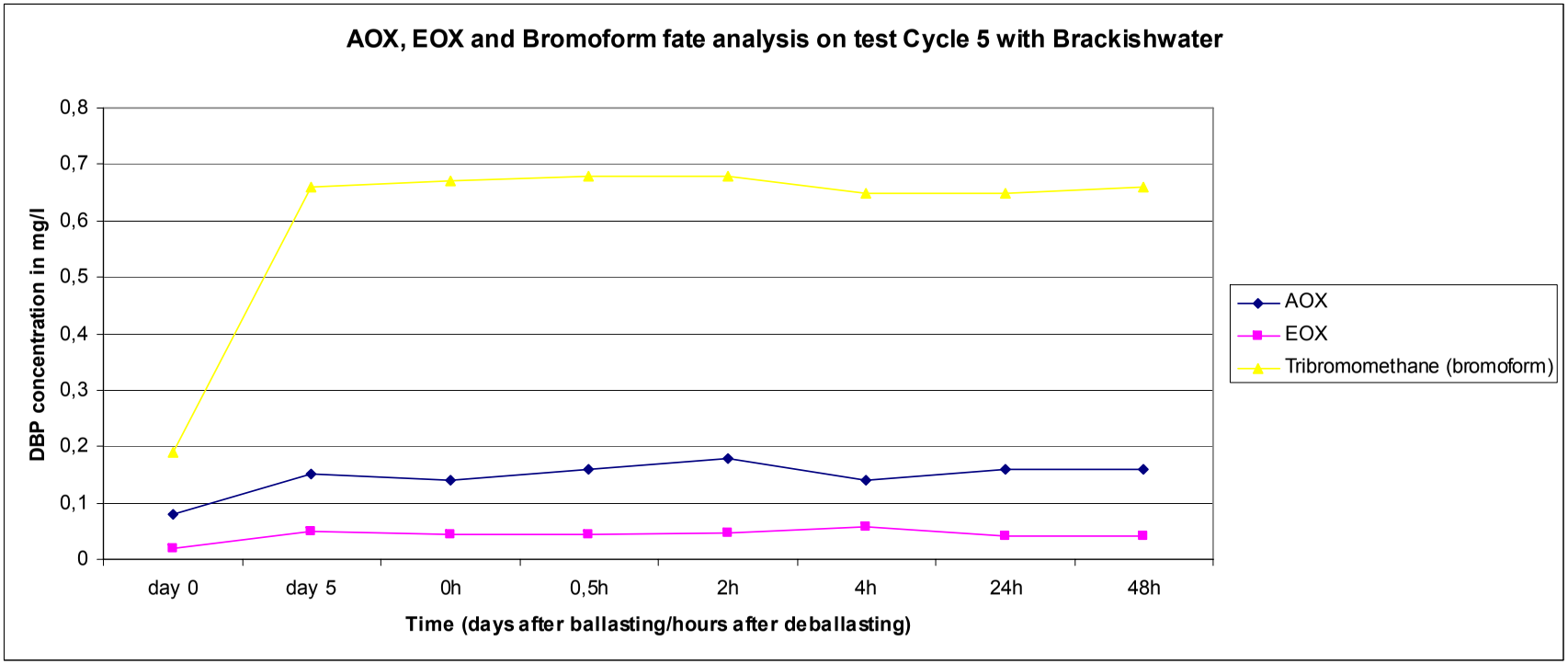

Figure 3 Concentration of disinfection by-products (mg/l) in treated brackish water in test cycle 5, measured at defined days after ballasting and defined hours after deballasting.

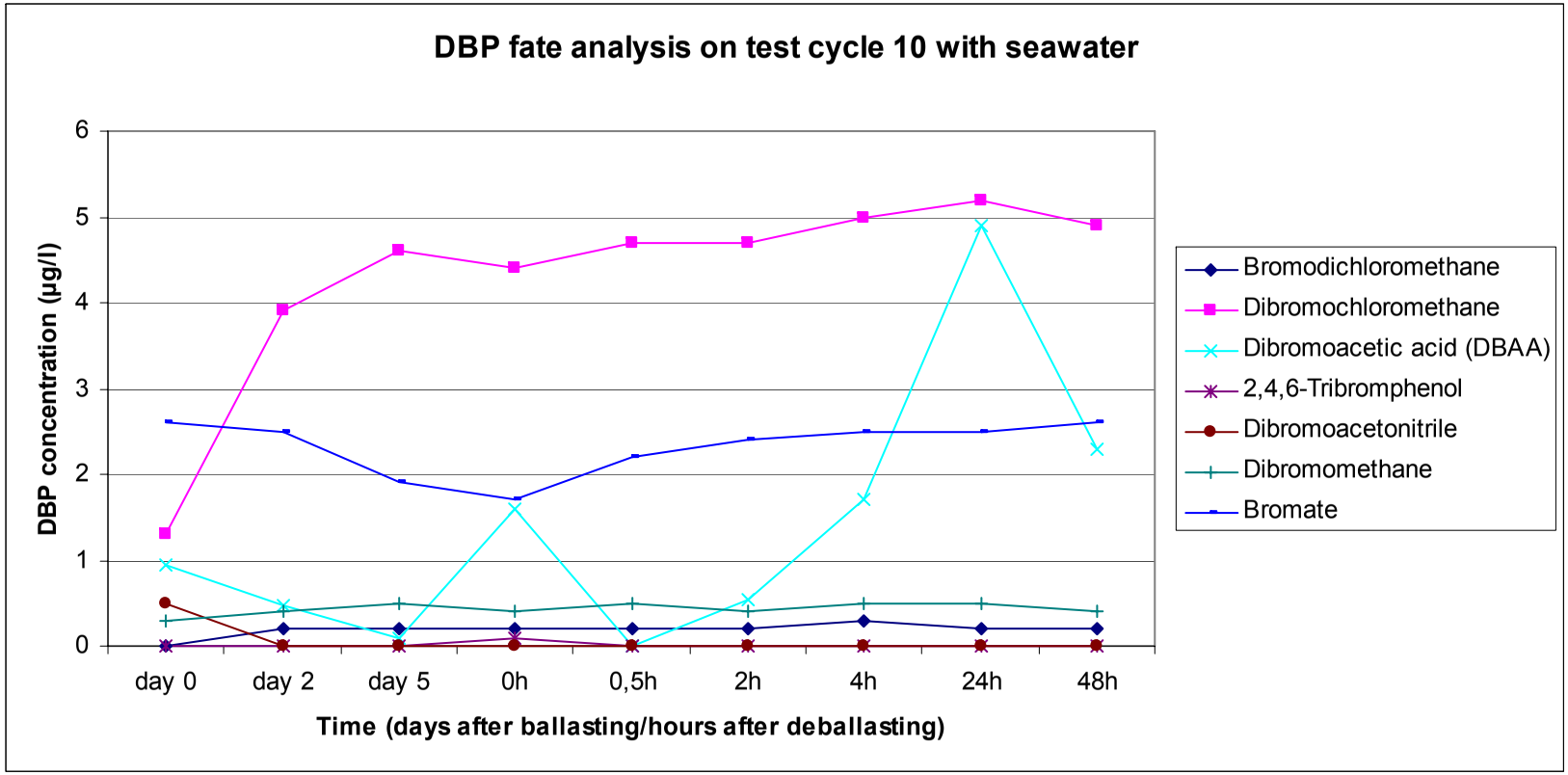

Figure 4 Concentration of disinfection by-products (µg/l) in treated seawater in test cycle 10, measured at defined days after ballasting and defined hours after deballasting.

The concentrations of the sum parameters AOX and EOX were surprisingly low compared to the concentrations of bromoform in the same test cycle (**Figure 3 and Figure 5**). There were relative small variations in organically bound halogens and extractable organically bound halogens during the time span, exept for AOX in seawater where the variations were significant (figure 5). All measurements of AOX and EOX were lower than the bromoform-concentrations. The analytical procedure for AOX and EOX may suffer from interference of some non-halogenated compounds in complex solutions such as seawater and brackish water. The shape of the curves for AOX and EOX were almost identical, except for AOX in seawater. AOX and EOX compounds are only analysed once and not reported with standard devation. It is therefore difficult to assess if this deviation is real or just within the variation of the analytical precision of the method.

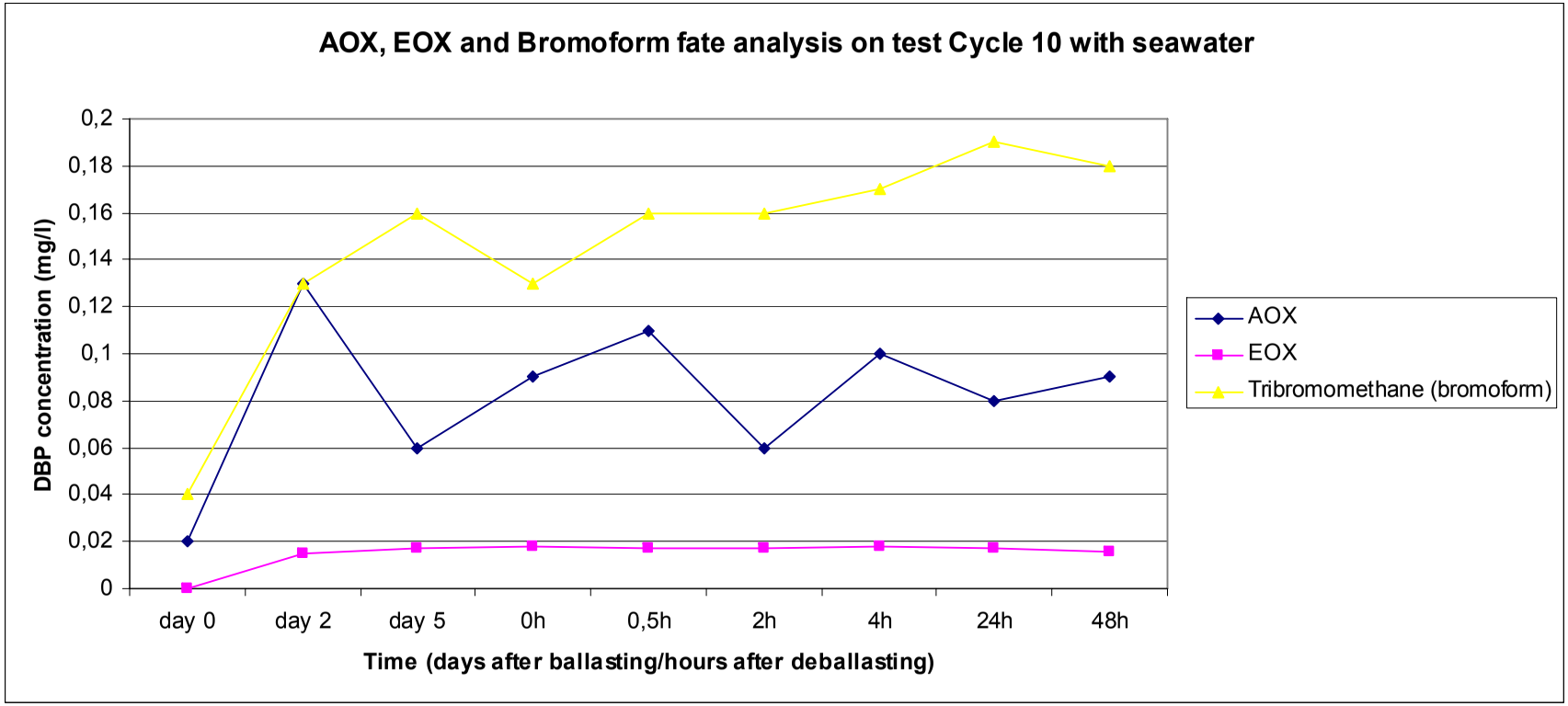

Figure 5 Concentration of disinfection by-products (mg/l) in treated seawater in test cycle 10, measured at defined days after ballasting and defined hours after deballasting.

The majority of bromoform was formed during the 2-5 first days (**Figure 3 and Figure 5**). The concentrations of bromoform detected were higher in brackish water than in seawater, and reached values between 600 µg/l - 700 µg/l. However, such levels are not higher than values that can be found in public swimming pools.

3.3.3 Characterization of water from filter backflushing

On day 0 in test cycles 5 (brackishwater) and 9 (seawater), backflush-water from the filter was sampled and analysed. Results are shown in **Table 12**.

Table12. Characterization of water from filter backflushing in test cyles 5 and 9.

Parameter	Unit	Cycle 5 Day 0	Cycle 9 Day 0
Temperature	ºC	nd	13.5
pH	PSU	8.05	8.0
Dissolved O_2	mg/l	8.7	8.1
Salinity	PSU	21.7	33.2
Redox	mV	nd	356
Total suspended solids (TSS)	mg/l	26.8	15.4
Total organic carbon (TOC)	mg/l	12.8	2.6
Dissolved organic carbon (DOC)	mg/l	6.5	2.7
Particulate organic carbon (POC)	mg/l	6.3	2.5
Ignition loss	%	38.8	42.9
Total dry matter* (or Settlings solids**)	g/l	24.0	51.98
Density*	g/ml	1.012	1.020
Turbidity	FNU	28.4	3.58

* Average of duplicate determinations

**The total amount of suspended material in the samples was under 10% of the total amount of solids. Therefore the determinations of total dry matter in the sample were conducted according to the method EN 12880 (as described in the paragraph 7.2.1 in NS EN 14702-1 for settlings solids determination)

3.3.4 Measurement of gasses in tank headspace

Measurements of head gas were performed during operation and storage of treated ballast water to assure the safety of test personell and equipment. The concentrations were measured just above the water surface inside the treated water tank during each treatment (day 0 and 5 with open cover) and each day between ballasting and deballasting (day 1 and 2 with cover closed).

The results from the table 13 show that only carbon monoxide (CO) gas and non-specific flammable gases at 65°C were detected. No hydrogen sulphide (H_2S) or chlorine (Cl_2) were detected. There were none of these gases detected above the hatch of the tank, during ballasting as well deballasting

CO was measured using a GasAlert MicroClip RW designed to react to concentrations ranges potentially harmful to humans and ship safety. Hence, the accuracy of the instrument is within the specification for this purpose,. Measurements were performed by keeping the sensor close to the water surface during treatment and at days 1, 2 and 5. For brackish water CO was detected at concentrations between 20 ppm and 358 ppm at any of these days, apparent with no regularity regarding at which day the highest concentration appeared. The same irregularity in observed peak was also the case for seawater, but at a somewhat lower level (12-138 ppm). This indicates that an effect of the treatment is increased CO – levels in the headspace gas.

Carbon monoxide (CO) is a toxic gas. Symptoms of mild poisoning include headaches and dizziness can occur at concentrations less than 50 ppm (ACGIH 1986). In the United States, NIOSH (National Institute for Occupational Safety and Health) has established a recommended exposure limit (REL) for carbon monoxide of 35 ppm (43 mg/m^3) as an 8-hour time-weighted average (TWA) and 200 ppm (247 mg/m^3) as a ceiling (NIOSH 1992). The MSDS sheet for CO is given in Appendix Q. CO is flammable within the concentration range of 12.5-74 % (1250-7400 ppm) and autoignites at 609 ^{o}C (NIOSH 1992).

A Dräger instrument with an x-am 5000 sensor was used to non-specifically measure all flammable gas at 65°C as % of LEL, which will include gasses as methane and hydrogen. Among flammable gases, it is known that hydrogen gas can be produced from electrolysis of water, hence, it is also a potential by-product of

electrocatalysis. The range for concentration of non-specific flammable gases at 65°C measured in the headspace for brackish water and seawater was 0-6% of LEL and 0-2.5% of LEL respectively, ref. table 13.

These same measurements were done in the control water tank also. None of the gases were detected here, so the results are not presented in the table 13. All results are given in appendix E.

The oxygen concentrations measured are presented in the raw data sheets in the appendix.

In summary, the measurements indicate elevated concentrations of CO above treated water, and some flammable gasses at low levels. More measurements with more sofisticated instruments could be undertaken to determine amounts of various gasses in the head gas.

Table 13: The highest levels of gases measure inside of the treated test water tank on day 0 after ballasting, day 1, day 2 and day 5 after deballasting given in ppm (parts per million) or % of LEL. Oxygen concentrations are reported in raw data.

Parameter	**CO** measured by Gas Alert instrument	**H_2S** measured by Gas Alert instrument	**Cl_2** measured by Dräger instrument	**Flammable gases at 65°C** measured by Dräger instrument
Unit	ppm	ppm	ppm	% of LEL
Test Cycle 1				
Day 0	108	0	0	3.5
Day 1	-	-	-	-
Day 2	-	-	-	-
Day 5	111	0	0	2.0
Test Cycle 2				
Day 0	110	0	0	-
Day 1	20	0	0	0
Day 2	-	-	-	-
Day 5	40	0	0	2.0
Test Cycle 3				
Day 0	70	0	0	2.0
Day 1	-	-	-	-
Day 2	-	-	-	-
Day 5	60	0	0	2.5
Test Cycle 4				
Day 0	358	0	0	6.0
Day 1	-	-	-	-
Day 2	-	-	-	-
Day 5	100	0	0	0
Test Cycle 5				
Day 0	100	0	0	3.0
Day 1	-	-	-	-
Day 2	-	-	-	-
Day 5	239	0	0	4.0
Test Cycle 6				
Day 0	32	0	0	2.0
Day 1	37	0	0	0
Day 2	111	0	0	2.5
Day 5	25	0	0	0
Test Cycle 7				
Day 0	43	0	0	0

Day 1	92	0	0	0
Day 2	114	0	0	2.5
Day 5	54	0	0	0
Test Cycle 8				
Day 0	30	0	0	0
Day 1	86	0	0	0
Day 2	100	0	0	0
Day 5	12	0	0	0
Test Cycle 9				
Day 0	23	0	0	0
Day 1	138	0	0	2.5
Day 2	44	0	0	0
Day 5	14	0	0	0
Test Cycle 10				
Day 0	24	0	0	0
Day 1	31	0	0	0
Day 2	82	0	0	0
Day 5	12	0	0	0
Test Cycle 11				
Day 0	100	0	0	2.5
Day 1	321	0	0	5.5
Day 2	175	0	0	3.5
Day 5	18	0	0	0
Test Cycle 12				
Day 0	53	0	-	-
Day 1	32	0	0	0
Day 2	128	0	0	2.5
Day 5	28	0	-	-
Test Cycle 13				
Day 0	82	0	-	-
Day 1	-	-	-	-
Day 2	-	-	-	-
Day 5	32	0	-	-

3.4 Fulfillment of the biological water quality criteria

The initial test waters' content of organisms should comply with the requirements in G8 as given in **Table 2.** Results from quantitative measurements of the initial test waters are given in **Table 14**.

Organisms ≥50 µm in minimum diameter

The requirements regarding density of the ≥50 µm group was met in all test cycles, except in test cycle 2 were the count was slightly lower than required. In this test cycle, samples for the ≥50 µm group were collected from the surface instead of from the bulk of the tank, due to human error, resulting in too low counts explained by sinking and swimming activities of *Artemia*. According to the amount of *Artemia* added, the average number per m^3 should be in compliance with G8. The requirements regarding the biological diversity within the population were fulfilled in all tests.

Organisms ≥10-50 µm in minimum diameter

The quantifications of individuals in the ≥10-50 µm group were based on growth in dilution series and microscope counts of CFDA-AM stained cells. In addition, growth on agar plates was used in some test cycles. The requirements regarding influent density of the ≥10-50 µm group was met in all tests cycles.

Heterotrophic bacteria

The only requirement regarding bacteria in influent water is that heterotrophic bacteria should be present in a density of $\geq 10^4$ cfu ml^{-1}. This requirement was fulfilled in all tests as shown in **Table 14.**

The IMO G8 guideline 2.3.20 also specifies that "…the following bacteria do not need to be added to the influent water, but should be measured at the influent and at the time of discharge: 1. Coliform; 2. Enterococcus group; 3. *Vibrio cholerae* and 4. Heterotrophic bacteria." All these bacteria groups were measured in the influent water and the results are given in **Table 14.**

Table 14. Initial content of organisms within the defined test organism groups (ref. **Table 2**) in the test water in test cycle 1-13. Green background indicates that required level was fulfilled, yellow background partial fulfilment, while red background indicates failure to fulfil required level.

Test organism	Method	Influent	Requirement
Test 1			
Organisms ≥50 µm	Microscope counts (cell/m3)	133 000±16 581	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	5000 2000-20000	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1 781±79	
	Plate counts (cell/ml)	1 650	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	$4.2 \pm 0.5 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	22 ± 24	-
Vibrio sp.	Bacterial counts (cfu/100ml)	$4.1 \pm 4.4 \times 10^5$	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	8 ± 6	-
Test 2			
Organisms ≥50 µm	Microscope counts (cell/m3)	76 179 ± 5582*	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	3000 1000-13000	≥1000 ml^{-1}
	Microscope counts (cell/ml)	2 265 ± 224	
	Plate counts (cell/ml)	1383	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	$2.0 \pm 0.1 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	23 ± 15	-
Vibrio sp.	Bacterial counts (cfu/100ml)	$1.6 \pm 0.2 \times 10^4$	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	3 ± 4	-
Test 3			
Organisms ≥50 µm	Microscope counts (cell/m3)	210 450 ± 32521	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	1600 600-5300	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1781 ± 210	
	Plate counts (cell/ml)	-	

	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	$9.0 \pm 1.2 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	4 ± 7	-
Vibrio sp.	Bacterial counts (cfu/100ml)	$1.3 \pm 0.53 \times 10^5$	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	33 ± 23	-
Test 4			
Organisms ≥50 µm	Microscope counts (cell/m3)	184 754 ± 40 044	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	2400 1000-9500	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1616 ± 388	
	Plate counts (cell/ml)	-	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	$2.5 \pm 0.36 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	<1	-
Vibrio sp.	Bacterial counts (cfu/100ml)	$4.8 \pm 1.7 \times 10^5$	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	17 ± 8	-
Test 5			
Organisms ≥50 µm	Microscope counts (cell/m3)	213 304 ± 26 383	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	5000 2 000-20 000	≥1000 ml^{-1}
	Microscope counts (cell/ml)	2 023 ± 187	
	Plate counts (cell/ml)	-	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	$> 3.5 \pm 1.0 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	<1	-
Vibrio sp.	Bacterial counts (cfu/100ml)	$7.8 \pm 0.5 \times 10^4$	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	37 ± 12	-
Test 6			
Organisms ≥50 µm	Microscope counts (cell/m3)	158 933 ± 22 648	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	2 400 1 000-9 500	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1 971 ± 280	
	Plate counts (cell/ml)	-	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	$>1.9 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	8 ± 3	-
Vibrio sp.	Bacterial counts (cfu/100ml)	$7.3 \pm 1.3 \times 10^3$	-

Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	19 ± 19	-
Test 7			
Organisms ≥50 µm	Microscope counts (cell/m3)	182 963 ± 23 328	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	1 300 500-3 900	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1 288 ± 260	
	Plate counts (cell/ml)	-	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	5.2 ± 0.5 x 10^4	≥10^4 cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	<1	-
Vibrio sp.	Bacterial counts (cfu/100ml)	7.6 ± 0.8 x 10^4	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	2 ± 0	-
Test 8			
Organisms ≥50 µm	Microscope counts (cell/m3)	213 708 ± 69 950	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	1300 500-3800	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1 288 ± 98	
	Plate counts (cell/ml)	-	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	1.8 ± 0.8 x 10^4	≥10^4 cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	<1	-
Vibrio sp.	Bacterial counts (cfu/100ml)	1.5 ± 1.0 x 10^3	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	82±22	-
Test 9			
Organisms ≥50 µm	Microscope counts (cell/m3)	173 883 ± 27 867	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	1300 500-3900	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1089 ± 182	
	Plate counts (cell/ml)	na	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	1.2 ± 0.1 x 10^4	≥10^4 cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	<1	-
Vibrio sp.	Bacterial counts (cfu/100ml)	8.4 ± 1.6 x 10^3	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	<1	-
Test 10			
Organisms	Microscope counts (cell/m3)	207475 ± 32969	≥100 000 m^{-3}

≥50 µm	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	2400 1000-9500	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1660 ± 225	
	Plate counts (cell/ml)	-	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	>1.4 ± 0.3 x 10^4	≥10^4 cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	7 ± 6	-
Vibrio sp.	Bacterial counts (cfu/100ml)	6.7 ± 1.1 x 10^3	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	9.4 ± 1.3 x 10^4	-
Test 11			
Organisms ≥50 µm	Microscope counts (cell/m3)	203483 ± 39734	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	3000 1000-13000	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1392 ± 83	
	Plate counts (cell/ml)	-	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	1.5 ± 0.4 x 10^4	≥10^4 cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	61 ± 14	-
Vibrio sp.	Bacterial counts (cfu/100ml)	1.1 ± 0.2 x 10^5	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	17 ± 9	-
Test 12			
Organisms ≥50 µm	Microscope counts (cell/m3)	161142 ± 17074	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	5000 2000-20000	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1478 ± 182	
	Plate counts (cell/ml)	-	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	>1.0 ± 0.04 x 10^4	≥10^4 cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	1 ± 1	-
Vibrio sp.	Bacterial counts (cfu/100ml)	1.8 ± 0.6 x 10^3	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	<1	-
Enterococcus group	Bacterial counts (cfu/100ml)	6 ± 6	-
Test 13			
Organisms ≥50 µm	Microscope counts (cell/m3)	163604 ± 18764	≥100 000 m^{-3}
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Organisms ≥10-50 µm	Dilution method 95 % conf. Interval (cell/ml)	2400 1000-13000	≥1000 ml^{-1}
	Microscope counts (cell/ml)	1348 ± 69	

	Plate counts (cell/ml)	-	
	Phyla	>3	≥3 different
	Species	>5	≥5 different
Marine heterotrophic bacteria	Bacterial counts (cfu/ml)	$4.5 \pm 0.6 \times 10^4$	$\geq 10^4$ cfu ml^{-1}
Coliform bacteria	Bacterial counts (cfu/ml)	<1	-
Vibrio sp.	Bacterial counts (cfu/100ml)	<1	-
Vibrio cholerae	Bacterial counts. Elimination method (cfu/100ml)	$4.1 \pm 0.2 \times 10^4$	-
Enterococcus group	Bacterial counts (cfu/100ml)	<1	-

*The count is slightly lower than the required ≥100 000 m^{-3}. The usual amount of *Artemia* was added, 1.2×10^8 hatched *Artemia*, which will give approximately 220 000 *Artemia*/m^3. However, in brackish water *Artemia* tend to sink/swim downwards which can give lower numbers in the surface. In test cycle 2, samples for the ≥50 µm group were collected from the surface instead of from the middle of the tank, resulting in too low counts.

3.5 Biocidal effects on organisms ≥50 µm in minimum diameter

The number of viable organisms ≥50 µm in minimum diameter, as determined on the basis of motility and integrity by microscope examination in treated test water and control immediately after treatment and after five days of storage, is given in **Table 15.**

For the performance of the ballast water treatment system to pass the regulation D-2 of the IMO guidelines, as shown in **Table 2,** less then 10 viable organisms ≥50 µm per m^3 should be present in the treated water after five days storage. This was met in all test cyles.

There were 2 cases where the number of viable organisms on day 0 in treated water was more than 10 (test cycle 2 and 13). In both these cases, an approximate 50 % reduction during the 5 days holding period was observed. A similar reduction has been observed in other studies and is believed to be an indication of that a ceratin fraction of the organism observed to be viable is in fact mortally injured and die during the holding period.

The equivalent requirements for the non treated water (control) is stated as: " If in any test the average discharge results from the control water is a concentration less then or equal to 10 times the values in regulation D-2.1, the test cycle is invalid." This has been interpreted to that the minimum level of viable organisms in the control water at the time of discharge (e.g after 5 days storage) should be higher than 100 organisms per m^3. This requirement was fulfilled in all tests.

Table 15. Viable organisms ≥50 µm in minimum diameter in treated test water and control immediately after treatment and after five days of storage. Green background indicates that the required level was fulfilled, yellow background partial fulfilment, while red background indicates failure to fulfil required level. (a.d. = after deballasting on day 5).

	Treated water		Control water	
	Day 0	**Day 5 (a.d)**	**Day 0**	**Day 5 (a.d.)**
Organisms ≥50 µm in minimum diameter (individuals m^{-3})				
Requirement	-	<10	-	>100
Test cycle 1	0	2.3±2.3	103 370±10 887	27 000 ±1 161
Test cycle 2*	18±6.6	7.7±1.2	68 780±8 532	19 444 ± 6 550
Test cycle 3	0.7±0.6	1.3±0.6	87 276±7 652	15 136 ± 4 783
Test cycle 4	4.7±0.6	0.7±0.6	64 241±24 015	7 331 ± 2 922
Test cycle 5	0.7±1.2	2.3±2.1	112 204± 11 765	28 589± 3574
Test cycle 6	0	1.3±0.6	119 467±16 572	88 413±8 162
Test cycle 7	4.0±2.6	0.3±0.6	131 828±13 401	85 785±4 416
Test cycle 8	1.7±0.6	0.3±0.6	125 370±28 598	89 671±4 943
Test cycle 9	0.7±0.6	0	75 961±12 978	76 068±10 045
Test cycle 10	0	1.7±2.1	126351±21 177	64129±9 099
Test cycle 11	8.6±2.3	4.6±1.5	128096±10106	63835±12026
Test cycle 12	3.6±3.5	2.6±1.2	98256±6898	52574±9723
Test cycle 13	15.3±11.1	8±1.7	97328±21122	38194±3470

*Technical failure

3.6 Biocidal effects on organisms ≥10-50 µm in minimum diameter

The number of viable organisms ≥10-50 µm in minimum diameter, as determined by the serial dilution method in algal growth medium and by microscopy examination after incubation with CFDA-AM in treated test water and control immediately after treatment, and after five days of storage, is given in **Table 16a** and **Table 16b**.

For the performance of the ballast water treatment system to pass the regulation D-2 of the IMO guidelines, as shown in **Table 2,** less than 10 viable organisms per ml should be present in the treated water after five days storage. The QHT BWMS fulfilled the requirements in most tests. However, as shown in **table 16a**, the results of the quantification of viable organisms in the size range 10-50 µm using the dilution method (MPN) show that the required level for test cycle 2 and 4 is exceeded. In test cycle 2, a total number of 24 organisms were estimated, while in test cycle 4 the number was estimated to 11.6 individuals, which is very close to the requirement of <10 organisms per ml. By inspection of the various alga species found, 8 individuals of the *Cryptomonas* group (size 9-12 µm) at day 5 were estimated in this test cycle (see Appendix 3). It was calculated that if only 2 individuals of these 8 were below 10 µm in size, the requirement would have been fulfilled.

In addition to the algae counts in table 16a, viable algal species with minimum diameter less than 10 µm were found in the test cycles 1 to 5. The most numerous species was *Emiliania huxleyi.* By microscopy inspection of seawater samples from Solbergstrand, a size range of 4.5-6 µm in minimum diameter was determined for this algae. Because the size was well below 10 µm, the cells were excluded from the counts shown in table 16a.

In table 16b, the results from staining with CFDA and observing the cells in microscope are shown. By this method, the required level was fulfilled in all test cycles. The method has problems to detect cells that have thin cell membranes as e.g. *Emiliania huxleyi* , so these small cells were probably excluded in the counts shown. As stated earlier, these cells were determined to have a minimum diameter well below 10 µm.

The equivalent requirements for the non treated water (control) is stated as: " If in any test the average discharge results from the control water is a concentration less then or equal to 10 times the values in regulation D-2.1, the test cycle is invalid." This has been interpreted to that the minimum level of viable organisms in the control water at the time of discharge (e.g after 5 days storage) should be higher than 100 organisms per ml.

This requirement was fulfilled in all tests, except test cycle 1 which was slightly below the required level. The initial density at day 0 was fulfilled (table 14).

Table 16a. Viable organisms ≥10-50 µm in minimum diameter in treated test water and control immediately after treatment and after five days of storage determined by the serial dilution method. Green background indicates that required level was fulfilled. (a.d.= after deballasting on day 5)

	Treated water		**Control water**	
	Day 0	**Day 5 (a.d.)**	**Day 0**	**Day 5 (a.d.)**
Organisms ≥10-50 µm in minimum diameter (individuals ml^{-1})				
Requirement	-	<10	-	>100
Dilution method 95 % confidence interval				
Test cycle 1	5.6	4.5	5000	90*
	3-19	2-17	2000-14000	30-300
Test cycle 2**	53	24	5000	160
	20-200	10-95	2000-14000	60-530
Test cycle 3	35	8.2	2000	500
	10-130	3-25	1000-14000	200-2000
Test cycle 4	14.4	11.6***	2000	220
	6-36	5-39	1000-14000	90-590
Test cycle 5	14.4	1.7	2000	900
	6-36	0.9-8.7	1000-14000	300-3000
Test cycle 6	0.7	<0.2	5000	>2400
	0.2-2.1	<0.1-1.0	2000-24000	1000-9500
Test cycle 7	<0.2	<0.2	2000	>2400
	<0.1-1.0	<0.1-1.0	1000-14000	1000-9500
Test cycle 8	0.2	0.4	2000	1300
	<0.1-1.0	0.1-1.5	1000-14000	500-3900
Test cycle 9	0.2	0.2	1300	1600
	<0.1-1.0	<0.1-1.0	500-3900	600-5300
Test cycle 10	<0.2	<0.2	2000	>2400
	<0.1-1.0	<0.1-1.0	1000-14000	1000-9500
Test cycle 11	<0.2	<0.2	2000	2400
	<0.1-1.0	<0.1-1.0	1000-14000	1000-9500
Test cycle 12	<0.2	<0.2	5000	>2400
	<0.1-1.0	<0.1-1.0	2000-24000	1000-9500
Test cycle 13	<0.2	<0.2	2000	>2400
	<0.1-1.0	<0.1-1.0	1000-14000	1000-9500

* The result obtained for the control water in this test cycle is close to the requirement of >100 organisms per ml.
** As mentioned in chapter 3.2, a technical failure in auxiliary equipment (air compressor, supplied by NIVA, for filter operation) occured during test cycle 2, which may have influenced the treatment efficiency and the biological This cycle should not be included in the biological efficiency evaluation of the BWMS with exception of the toxicity data retrieved from this cycle, as the concentration of active substance was not influenced by the technical failure.
*** The result obtained in this test cycle is very close to the requirement of <10 organisms per ml. This issue is more discussed in Appendix 3.

Table 16b. Viable organisms ≥10-50 µm in minimum diameter in treated test water and control immediately after treatment and after five days of storage determined by microscopy examination after incubation with CFDA-AM. Green background indicates that required level was fulfilled. (a.d.= after deballasting on day 5).

Microscope counts				
	Treated water		**Control water**	
	Day 0	**Day 5 (a.d.)**	**Day 0**	**Day 5 (a.d.)**
Test cycle 1	3.3±2.3	2.0±1.0	1305±15	70±30
Test cycle 2*	4.0±2.0	2.3±1.0	2316±261	484±24
Test cycle 3	7.7±2.1	6.0±1.0	2023±225	1046±208
Test cycle 4	12.3±8.5	5.7±2.9	1383±221	135±12
Test cycle 5	3.7±0.6	0.3±0.6	2187±378	2394±188
Test cycle 6	0	0	2230±69	2066±241
Test cycle 7	0	0	1832±75	1729±328
Test cycle 8	0	0	1755±143	1746±198
Test cycle 9	0	0	1642±234	2005±65
Test cycle 10	0	0	1487±177	2299±108
Test cycle 11	0	0	1426±299	1478±226
Test cycle 12	0	0	1962±152	2031±195
Test cycle 13	0	0	1418±15	1521±117

* Technical failure

3.7 Bactericidal effects

The numbers of heterotrophic bacteria, coliform bacteria, *E.coli, Vibrio* sp., *Vibrio cholerae, Enterococcus* group and intestinal *Enterococci*, determined in treated water and control water immediately after treatment and after five days of storage are given in **Table 17**. Regulation D-2 only requires documentation of the bactericidal effect of the ballast water treatment system on *E.coli*, *Vibrio cholera* (toxicogenic serotypes O1 and 0139) and intestinal *Enterococi* given as maximum allowable concentration in discharge waters of < 250 cfu/100 ml, < 1 cfu/100 ml and < 100 cfu/100 ml, respectively. These requirements were fulfilled for all bacterial species in all test cycles. *Vibrio* sp. is common in salt and brackish surface water. However, the possibility of further detection of the serotypes O1 and O139 is very small or absent since none of these serotypes have been isolated from Norwegian coastal water. *Vibrio cholera* was not found in any of the samples from treated water during the 13 test cycles.

Table 17. Heterotrophic bactera, coliform bacteria, *E.coli, Vibrio* sp. *Vibrio cholera*, Enterococcus group and intestinal *Enterococci* in treated test water and control water immediately after treatment and after five days of storage for test cycles 1-13. Green background indicates that required level was fulfilled. (a.d.= after deballasting on day 5).

	Treated water			**Control water**	
	Day 0	**Day 5 (a.d.)**		**Day 0**	**Day 5 (a.d.)**
Marine heterotrophic bacteria (cfu ml^{-1})					
Requirement	-	-		-	-
Test cycle 1	$2.1 \pm 1.4 \times 10^{2}$	$> 1.0 \times 10^{6}$		$2.6 \pm 0.6 \times 10^{4}$	$4.5 \pm 1.0 \times 10^{5}$
Test cycle 2[1]	$6.6 \pm 4.4 \times 10^{0}$	$9.4 \pm 1.6 \times 10^{5}$		$5.0 \pm 2.1 \times 10^{4}$	$2.5 \pm 0.4 \times 10^{5}$
Test cycle 3	$9.9 \pm 3.2 \times 10^{0}$	$1.5 \pm 0.1 \times 10^{6}$		$1.2 \pm 0.2 \times 10^{5}$	$2.4 \pm 0.1 \times 10^{5}$
Test cycle 4	$4.1 \pm 0.6 \times 10^{1}$	$1.2 \pm 0.9 \times 10^{6}$		$1.9 \pm 0.3 \times 10^{4}$	$1.5 \pm 0.6 \times 10^{5}$
Test cycle 5	$2.7 \pm 2.1 \times 10^{1}$	$1.9 \pm 2.4 \times 10^{6}$		$3.5 \pm 1.0 \times 10^{4}$	$2.3 \pm 0.4 \times 10^{5}$
Test cycle 6	$8.7 \pm 8.5 \times 10^{1}$	$1.1 \pm 0.2 \times 10^{5}$		$9.8 \pm 2.1 \times 10^{3}$	$2.2 \pm 0.6 \times 10^{5}$
Test cycle 7	$6.0 \pm 5.2 \times 10^{0}$	$4.7 \pm 2.6 \times 10^{3}$		$2.8 \pm 0.0 \times 10^{4}$	$1.2 \pm 0.0 \times 10^{5}$
Test cycle 8	<1	$3.7 \pm 2.3 \times 10^{2}$		$1.7 \pm 0.3 \times 10^{4}$	$4.3 \pm 0.8 \times 10^{5}$
Test cycle 9	$0.9 \pm 1.3 \times 10^{0}$	$1.2 \pm 0.2 \times 10^{3}$		$1.0 \pm 0.5 \times 10^{4}$	$4.4 \pm 0.9 \times 10^{4}$
Test cycle 10	$4.5 \pm 6.4 \times 10^{0}$	$3.5 \pm 0.2 \times 10^{2}$		$1.4 \pm 0.3 \times 10^{4}$	$3.3 \pm 0.4 \times 10^{5}$
Test cycle 11	$0.3 \pm 0.5 \times 10^{0}$	$3.7 \pm 0.6 \times 10^{4}$		$1.7 \pm 0.4 \times 10^{4}$	$1.1 \pm 0.1 \times 10^{6}$
Test cycle 12	<1	$7.2 \pm 3.2 \times 10^{3}$		$1.0 \pm 0.0 \times 10^{4}$	$7.0 \pm 1.3 \times 10^{5}$
Test cycle 13	$1.5 \pm 1.9 \times 10^{0}$	$2.9 \pm 1.2 \times 10^{4}$		$3.9 \pm 0.6 \times 10^{4}$	$3.0 \pm 0.6 \times 10^{5}$
Coliform bacteria (Coli.) and *Escherichia coli (E. coli) (cfu 100 ml^{-1})**					
	Coli.	**Coli.**	***E. coli***	**Coli.**	**Coli.**
Requirement	-	-	<250*	-	-
Test cycle 1	<1	<1	<1	12 ± 7	<1
Test cycle 2[1]	<1	3 ± 6	<1	40 ± 10	2 ± 1
Test cycle 3	<1	<1	<1	2 ± 3	11 ± 1
Test cycle 4	<1	<1	<1	<1	<1
Test cycle 5	<1	<1	<1	<1	<1
Test cycle 6	<1	1 ± 1	<1	<1	1 ± 1
Test cycle 7	9 ± 3	<1	<1	<1	<1
Test cycle 8	<1	<1	<1	<1	<1
Test cycle 9	<1	<1	<1	<1	<1
Test cycle 10	<1	<1	<1	<1	<1
Test cycle 11	<1	<1	<1	71 ± 27	<1
Test cycle 12	<1	<1	<1	<1	<1
Test cycle 13	<1	<1	<1	<1	<1

Vibrio* sp. and *Vibrio cholerae (*V. cholerae*) (cfu 100 ml^{-1})**					
	***Vibrio* sp.**	***Vibrio* sp**	***V. cholerae***	***Vibrio* sp.**	***Vibrio* sp.**
Requirement	-	-	<1**	-	-
Test cycle 1	$2.4 \pm 3.4 \times 10^{1}$	$> 1.0 \times 10^{6}$	<1	$2.4 \pm 2.3 \times 10^{5}$	$> 1.0 \times 10^{6}$
Test cycle 2[1]	$1.5 \pm 1.0 \times 10^{1}$	$1.6 \pm 0.1 \times 10^{6}$	<1	$3.4 \pm 1.4 \times 10^{4}$	$5.5 \pm 1.7 \times 10^{5}$
Test cycle 3	$2.5 \pm 1.0 \times 10^{1}$	$1.2 \pm 0.1 \times 10^{6}$	<1	$1.3 \pm 0.5 \times 10^{5}$	$9.2 \pm 0.92 \times 10^{5}$
Test cycle 4	$2.3 \pm 1.0 \times 10^{2}$	$2.6 \pm 1.9 \times 10^{6}$	<1	$3.2 \pm 0.5 \times 10^{5}$	$7.3 \pm 1.9 \times 10^{6}$
Test cycle 5	$4.7 \pm 3.8 \times 10^{0}$	$5.4 \pm 4.0 \times 10^{5}$	<1	$7.1 \pm 3.6 \times 10^{4}$	$5.0 \pm 1.7 \times 10^{5}$
Test cycle 6	<1	$1.8 \pm 1.0 \times 10^{3}$	<1	$1.6 \pm 0.4 \times 10^{4}$	$1.5 \pm 0.6 \times 10^{4}$
Test cycle 7	$2.1 \pm 1.9 \times 10^{1}$	$7.6 \pm 3.3 \times 10^{2}$	<1	$5.9 \pm 0.5 \times 10^{4}$	$4.9 \pm 1.2 \times 10^{4}$
Test cycle 8	$2.7 \pm 1.6 \times 10^{0}$	$1.0 \pm 1.2 \times 10^{2}$	<1	$2.1 \pm 0.5 \times 10^{4}$	$1.4 \pm 0.1 \times 10^{5}$
Test cycle 9	<1	$3.2 \pm 2.8 \times 10^{2}$	<1	$9.7 \pm 3.2 \times 10^{3}$	$4.0 \pm 2.0 \times 10^{4}$
Test cycle 10	$1.1 \pm 0.3 \times 10^{1}$	$7.0 \pm 2.8 \times 10^{1}$	<1	$6.8 \pm 1.0 \times 10^{3}$	$4.9 \pm 2.8 \times 10^{4}$
Test cycle 11	$0.6 \pm 1.0 \times 10^{0}$	$4.9 \pm 1.6 \times 10^{1}$	<1	$1.0 \pm 0.3 \times 10^{5}$	$2.0 \pm 0.4 \times 10^{5}$
Test cycle 12	$5.1 \pm 1.4 \times 10^{0}$	$7.6 \pm 1.9 \times 10^{1}$	<1	$2.1 \pm 0.3 \times 10^{3}$	$1.5 \pm 0.2 \times 10^{4}$
Test cycle 13	$2.7 \pm 0.6 \times 10^{1}$	$7.6 \pm 0.9 \times 10^{2}$	<1	$3.7 \pm 0.9 \times 10^{4}$	$5.3 \pm 3.9 \times 10^{4}$
Enterococcus group (Ent. gr.) and Intestinal *Enterococci* (Int. *Ent.*)* (cfu 100 ml^{-1})**					
	Ent. gr.	**Ent. gr.**	**Int. *Ent.***	**Ent. gr.**	**Ent. gr.**
Requirement	-	-	<100***	-	-
Test cycle 1	<1	26 ± 11	<1	24 ± 8	19 ± 15
Test cycle 2[1]	<1	5 ± 6	<1	21 ± 17	23 ± 24
Test cycle 3	<1	18 ± 3	<1	49 ± 1	18 ± 5
Test cycle 4	1 ± 1	3 ± 3	0.3 ± 1	28 ± 3	4 ± 0
Test cycle 5	<1	1 ± 1	<1	37 ± 12	17 ± 3
Test cycle 6	0.3 ± 1	1 ± 2	0.3 ± 1	69 ± 1	91 ± 69
Test cycle 7	<1	1 ± 1	<1	6 ± 2	49 ± 32
Test cycle 8	<1	<1	<1	120 ± 10	168 ± 149
Test cycle 9	<1	<1	<1	<1	20 ± 6
Test cycle 10	<1	<1	<1	12 ± 3	66 ± 44
Test cycle 11	<1	7 ± 2	<1	17 ± 8	18 ± 4
Test cycle 12	<1	4 ± 6	<1	6 ± 2	3 ± 3
Test cycle 13	<1	<1	<1	<1	<1

[1]Technical failure

* The figures refer to the number identified as *Escherichia coli*/100 ml within the group of coliform bacteria. There is a requirement for *Escherichia coli* being <250 cfu/100ml after five days storage (D-2).

** The figures refer to the number identified as *Vibrio cholerae*/100 ml after five days storage. There is a requirement for toxicogenic *Vibrio cholerae* (serotypes O1 and O139) being <1 cfu/100 ml after five days storage (D-2).

*** The figures refer to the number identified as intestinal *Enterococci*/100 ml within the group of Enterococcus. There is a requirement for intestinal *Enterococci* being <100 cfu/100ml after five days storage (D-2).

3.8 Ecotoxicological responses

Fish toxicity testing
Both acute and chronic test were performed on both brakish water and seawater. It was no observations of toxic effects in any of the tests.

Table 18. Summary of the results for the fish test performed; acute and chronic tests with juvenile turbot (*Scopthalmus maximus*).

Fish Acute tests				
Test cycle	**Test id**	**Test sample**	**Result LC50 (%)**	**Comment**
Test 2*	B655		>100 %	
Test 6	B670		>100 %	
Fish Chronic test				
			Result NOEC (%)	
Test 2-5	B655		≥100 %	
Test 7-10	B673		≥100 %	

* It was observed a technical failure in auxiliary equipment (air compressor for filter operation) during test cycle 2, which may have influenced the treatment efficiency and the biological data with exception of the toxicity data retrieved from this cycle, as the concentration of active substance was not influenced by the technical failure.

Invertebrate toxicity testing
The invertebrate tests were performed using the copepod *Acartia tonsa*. This is a fairly sensitive species. No toxic effects were observed in any of the acute tests. In test cycle 11 performed with a higher initial oxidant concentration, also no effects were observed, indicating a margin of safety with respect to the oxidant levels used in test cycle 1-5.

Table 19. Test results of toxicity testing of ballast water treated with QHT BWMS tested with invertebrates; acute toxicity to *Acartia tonsa* and reproductive toxicity to *Nitocra spinipes*.

Acute Invertebrate tests				
Test Cycle	**Test id**	**Test sample**	**Result NOEC %**	**Comment**
Test 2*	B655		>100 %	
Test 6	B670		>100 %	
Test 11	B684		>100 %	
Chronic Invertebrate tests				
Test 1-2	B652-B655		≥100 %	
Test 7-8	B673-B676		≥100 %	

* It was observed a technical failure in auxiliary equipment (air compressor for filter operation) during test cycle 2, which may have influenced the treatment efficiency and the biological data with exception of the toxicity data retrieved from this cycle, as the concentration of active substance was not influenced by the technical failure.

Growth inhibition of the marine alga *Skeletonema costatum*

A total of 31 algal growth inhibition tests were performed on QHT BWMS treated water. The results are presented in Table 20 with EC10 as the chronic endpoint and with EC50 as the acute endpoint. In general, only a small acute or chronic effect was observed on day 0. This effect was reduced or disappeared on day 5. The average EC10 on day 0 for all brackish water tests was 51 % and the average EC50=73 % for the same tests. Similar values for full seawater tests is an average EC10=42 % and an average EC50=50 %.

For treated water on day 5, effects were only observed in one out of 13 tests. For the chronic endpoint, a measurable EC10 was only observed in 6 of 13 test cycles. In test cycle 5 and test cycle 10, a time series were conducted where additional test were performed on day 1 and 2. In test 5 we did not see a significant increase in EC50 and EC10 on day 1 and day 2, as may be expected as the day 5 sample showed no effect on algae. One should be aware that the growth inhibition test performed on day 1 and day 2 sample was from a sample stored in a glass bottle in the dark at 4 ºC, and therefore may not be representative of reactions occurring in the 200 m^3 tank at a higher temperature.

There seems to be a weak correlation between measurable values of TRO on day 5 (Table 9) and effect on algae in the growth inhibition tests.

Table 20. Effect concentrations (EC10 and EC50) as percentage treated ballast water diluted in control water.

Test cycle	Day nr	Date	EC10 (%)	EC50 (%)
1	0	22.7	23	>100
	5	27.7	>100	>100
2*	0	29.7	35	>100
	5	3.8	96	>100
3	0	5.8	56	61
	5	10.8	49	54
4	0	12.8	100	>100
	5	17.8	>100	>100
5	0	19.8	52	58
	1	20.8	56	61
	2	21.8	53	59
	5	24.8	>100	>100
6	0	2.9	34	43
	5	7.9	>100	>100
7	0	9.9	37	49
	5	14.9	80	>100
8	0	16.9	52	56
	5	21.9	97	>100
9	0	23.9	34	43
	5	28.9	>100	>100
10	0	30.9	55	57
	1	1.10	77	>100
	2	2.10	>100	>100
	5	5.10	>100	>100
	7	7.10	>100	>100
11	0	7.10	53	60
	5	12.10	68	>100
12	0	14.10	36	46
	5	19.10	>100	>100
13	0	21.10	52	57
	5	26.10	68	>100

* It was observed a technical failure in auxiliary equipment (air compressor for filter operation) during test cycle 2, which may have influenced the treatment efficiency and the biological data with exception of the toxicity data retrieved from this cycle, as the concentration of active substance was not influenced by the technical failure.

Oyster early life stage test

The oyster larvae test is a sensitive test, were it can be difficult to be sure if the observed effects are treatment related or is due to general water quality of the test water. In the test we have performed we have used aged seawater as control and for diluting the treated water, while untreated ballast water is tested separately. In both test cycle 3 and 13 were the test water is brackish water, we observed no survival of larvae in untreated ballast

water. One possible explanation for the higher toxicity of untreated water may be presence of higher numbers of bacteria which can be pathogenic to the oyster larvae. High amounts of dissolved organic matter in the brackish water tests may result in growth of such bacteria. It is therefore concluded that the the observed effects in treated ballst water in test cycle 3 and 13 can not be claimed to be caused by the BWMS, since the effects in untreated waters are higher.

Table 21. Test results of toxicity testing of ballast water treated with QHT BWMS using oyster embryo.

Chronic oyster embyo tests					
Test Cycle	**Test id**	**Test sample**	**Result LC50 %**	**Result NOEC (%)**	**Comment**
Test 3	B663		100 %	56 %	Untreated water showed more effects*
Test 8	B676		>100 %	≥100 %	
Test 13	B691		52 %	32 %	Untreated water had similar results**

* and **, in these test the untreated ballast water showe higher mortality (*) or equal mortality (**) to that of the untreated ballast water.

Reproduction test with rotatoria *Brachionus plicatilis*

The reproduction of *B. plicatilis* was tested both in brackish water and seawater at 5 concentrations in the range of 10 to 100 % dilutions of treated ballast water after discharge (**Table 22**). Statistical assessment of the observed reproduction indicated no significant effects.

Table 22. Test results of toxicity testing of ballast water treated with QHT BWMS using *Brachionus plicatilis.*

Chronic rotifer tests					
Test Cycle	**Test id**	**Test sample**	**Result EC50 (%)**	**Result NOEC (%)**	**Comment**
Test 4	B663		>100 %	≥100 %	
Test 10	B682		>100 %	≥100 %	

3.9 Summary and conclusion with respect to toxicity of treated ballast water on discharge

A total of 45 toxicity tests with 6 different species and 5 different phyla have been performed. In the growth inhibition tests with algae with discharge water on day 5, effect was only observed in one out of 13 tests. In acute and chronic tests with juvenile turbot (*Scopthalmus maximus*), no observations of toxic effects were observed. In the invertebrate tests performed using the copepod *Acartia tonsa*, no toxic effects were observed in any of the tests. The reproduction of *B. plicatilis* was tested both in brackish water and seawater at 5 concentrations of treated ballast water after discharge. Statistical assessment of the reproduction indicated no significant effects.. In the oyster larvae test, the observed toxic effects on the larvae in treated ballast water in two test cycles could not be claimed to be caused by the BWMS, since the toxic effects in untreated waters was higher, possible due to toxic effects of remaining bacteria.

The results of the toxicity testing indicate that treatment with QHT BWMS produce ballast water with little or no toxic effects upon discharge. It is therefore unlikely that the treated and discharged ballast water will have any adverse effect in the recipient water upon deballasting.

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Appendix 1 - Disinfection by-product analyses

Table 1 Disinfection by-products analysis in treated and control brackish water in test cycle 1.

Disinfection by-products	Unit	Detection limit	Influent	Treated		Control	
Time (day)			0	0	5	0	5
Parameter							
Test Cycle 1 (Salinity <22 PSU)							
Trichloromethane (chloroform)	µg/l	0.1	<0.10	<0.10	0.1	<0.10	<0.10
Bromodichloromethane	µg/l	0.1	<0.10	**0.4**	0.5	<0.10	<0.10
Dibromochlorormetane	µg/l	0.1	<0.10	**11**	12	<0.10	<0.10
Tribromomethane (bromoform)	µg/l	0.1	<0.10	**230**	290	<0.10	<0.10
Chloroacetic acid (MCAA)	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)	µg/l	0.3	<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)	µg/l	0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Bromoacetic acid (MBAA)	µg/l	0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)	µg/l	0.1	<0.10	**5.1**	<0.10	<0.10	<0.10
Bromochloroacetic acid (BCAA)	µg/l	0.1	<0.10	**0.13**	<0.10	<0.10	<0.10
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacetic acid (TBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dichloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Trichloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,4-Dibromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-Dibromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-Tribromophenol	µg/l	0.1	<0.10	**0.1**	<0.10	<0.10	<0.10
1,2-Dibromoethane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,4-Tribromobenzene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3-Trichloropropane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2-Chlorotoluene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
4-Chlorotoluene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2-Dibromo-3-chloropropane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3-Tribromobenzene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,3,5-Tribromobenzene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Monobromoacetonitrile	µg/l	0.1	<0.10	**0.6**	<0.10	<0.10	<0.10
Dibromoacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Bromochloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromomethane	µg/l	0.1	<0.10	<0.10	**1.3**	<0.10	<0.10
AOX	mg/l	0.01	**0.03**	**0.11**	**0.11**	**0.03**	**0.03**
EOX	mg/l	0.01	**<0.010**	**0.018**	**0.024**	<0.010	<0.01
Bromate	µg/l	1.0	<1.0	**6.0**	**6.0**	<1.0	<1.0

Table 2 Disinfection by-products analysis in treated and control brackish water in test cycle 4

Disinfection by-products	Unit	Detection limit	Influent	Treated		Control	
Time (day)			**0**	**0**	**5**	**0**	**5**
Parameter							
Test Cycle 4 (Salinity <22 PSU)							
Trichloromethane (chloroform)	µg/l	0.1	<0.10	<0.10	**0.1**	<0.10	<0.10
Bromodichloromethane	µg/l	0.1	<0.10	**0.2**	**0.7**	<0.10	<0.10
Dibromochlorormetane	µg/l	0.1	<0.10	**10**	**15**	<0.10	<0.10
Tribromomethane (bromoform)	µg/l	0.1	<0.10	**280**	**380**	<0.10	<0.10
Chloroacetic acid (MCAA)	µg/l	0.5	<0.50	<0.50	-	<0.50	-
Dichloroacetic acid (DCAA)	µg/l	0.3	<0.30	<0.30	-	<0.30	-
Trichloroacetic acid (TCAA)	µg/l	0.2	<0.20	<0.20	-	<0.20	-
Bromoacetic acid (MBAA)	µg/l	0.2	<0.20	<0.20	-	<0.20	-
Dibromoacetic acid (DBAA)	µg/l	0.1	<0.10	**9.6**	**3.1**	<0.10	<0.10
Bromochloroacetic acid (BCAA)	µg/l	0.1	<0.10	**0.34**	**0.3**	<0.10	<0.10
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	<0.10	<0.10	-	<0.10	-
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	<0.10	<0.10	-	<0.10	-
Tribromoacetic acid (TBAA)	µg/l	0.1	<0.10	<0.10	-	<0.10	-
Dichloroacetonitrile	µg/l	0.1	<0.10	<0.10	-	<0.10	-
Trichloroacetonitrile	µg/l	0.1	<0.10	<0.10	-	<0.10	-
2,4-Dibromophenol	µg/l	0.1	<0.10	<0.10	-	<0.10	-
2,6-Dibromophenol	µg/l	0.1	<0.10	<0.10	-	<0.10	-
2,4,6-Tribromophenol	µg/l	0.1	<0.10	<0.10	-	<0.10	-
1,2-Dibromoethane	µg/l	0.1	<0.10	<0.10	-	<0.10	-
1,2,4-Tribromobenzene	µg/l	1.0	<1.0	<1.0	-	<1.0	-
1,2,3-Trichloropropane	µg/l	0.1	<0.10	<0.10	-	<0.10	-
2-Chlorotoluene	µg/l	0.1	<0.10	<0.10	-	<0.10	-
4-Chlorotoluene	µg/l	0.1	<0.10	<0.10	-	<0.10	-
1,2-Dibromo-3-chloropropane	µg/l	0.1	<0.10	<0.10	-	<0.10	-
1,2,3-Tribromobenzene	µg/l	1.0	<1.0	<1.0	-	<1.0	-
1,3,5-Tribromobenzene	µg/l	1.0	<1.0	<1.0	-	<1.0	-
Monobromoacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromoacetonitrile	µg/l	0.1	<0.10	<0.10	-	<0.10	-
Bromochloroacetonitrile	µg/l	0.1	<0.10	<0.10	-	<0.10	-
Dibromomethane	µg/l	0.1	<0.10	**0.2**	<0.10	<0.10	<0.10
AOX	mg/l	0.01	<0.010	**0.13**	**0.1**	<0.010	<0.010
EOX	mg/l	0.01	<0.010	**0.022**	**0.034**	<0.010	<0.010
Bromate	µg/l	1.0	<1.0	**5.5**	**4.4**	<1.0	<1.0

Table 3 Disinfection by-products analysis in treated and control brackish water in test cycle 5

Disinfection by-products	Unit	Detection limit	Influent	Treated		Control	
Time (day)			**0**	**0**	**5**	**0**	**5**
Parameter							
Test Cycle 5 (Salinity <22 PSU)							
Trichloromethane (chloroform)	µg/l	0.1	-	**0.1**	**0.1**	<0.10	<0.10
Bromodichloromethane	µg/l	0.1	-	**0.4**	**0.7**	<0.10	<0.10
Dibromochlorormetane	µg/l	0.1	-	**7.4**	**18**	<0.10	<0.10
Tribromomethane (bromoform)	µg/l	0.1	-	**190**	**670**	<0.10	<0.10
Dibromoacetic acid (DBAA)	µg/l	0.1	-	**12**	**3.1**	**0.1**	**0.1**
Bromochloroacetic acid (BCAA)	µg/l	0.1	-	**0.41**	**0.18**	<0.10	<0.10
Monobromoacetonitrile	µg/l	0.1	-	<0.10	<0.10	<0.10	<1.0
Dibromomethane	µg/l	0.1	-	<0.10	<0.10	<0.10	<0.10
AOX	mg/l	0.01	-	**0.08**	**0.14**	<0.010	<0.010
EOX	mg/l	0.01	-	**0.02**	**0.045**	<0.010	<0.010
Bromate	µg/l	1.0	-	**7.2**	**6.6**	<1.0	<1.0

Table 4 Disinfection by-products analysis in treated and control seawater in test cycle 6.

Disinfection by-products	Unit	Detection limit	Influent	Treated		Control	
Time (day)			**0**	**0**	**5**	**0**	**5**
Parameter							
Test Cycle 6 (Salinity >32 PSU)							
Trichloromethane (chloroform)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Bromodichloromethane	µg/l	0.1	<0.10	<0.10	**0.4**	<0.10	<0.10
Dibromochlorormetane	µg/l	0.1	<0.10	**1.7**	**5.7**	<0.10	<0.10
Tribromomethane (bromoform)	µg/l	0.1	<0.10	**52**	**170**	<0.10	<0.10
Chloroacetic acid (MCAA)	µg/l	0.5	<0.50	<0.50	<0.50	<0.50	<0.50
Dichloroacetic acid (DCAA)	µg/l	0.3	<0.30	<0.30	<0.30	<0.30	<0.30
Trichloroacetic acid (TCAA)	µg/l	0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Bromoacetic acid (MBAA)	µg/l	0.2	<0.20	<0.20	<0.20	<0.20	<0.20
Dibromoacetic acid (DBAA)	µg/l	0.1	**0.52**	**26**	**8.5**	**0.21**	<0.20
Bromochloroacetic acid (BCAA)	µg/l	0.1	<0.10	**0.31**	**0.11**	<0.10	<0.10
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Tribromoacetic acid (TBAA)	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dichloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Trichloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,4-Dibromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,6-Dibromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2,4,6-Tribromophenol	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2-Dibromoethane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,4-Tribromobenzene	µg/l	0.1	<1.0	<1.0	<1.0	<1.0	<1.0
1,2,3-Trichloropropane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
2-Chlorotoluene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
4-Chlorotoluene	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2-Dibromo-3-chloropropane	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
1,2,3-Tribromobenzene	µg/l	0.1	<1.0	<1.0	<1.0	<1.0	<1.0
1,3,5-Tribromobenzene	µg/l	0.1	<1.0	<1.0	<1.0	<1.0	<1.0
Monobromoacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromoacetonitrile	µg/l	0.1	<0.10	<0.10	**0.8**	<0.10	<0.10
Bromochloroacetonitrile	µg/l	0.1	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromomethane	µg/l	0.1	<0.10	<0.20	**0.6**	<0.10	<0.10
AOX	mg/l	0.01	<0.010	**0.01**	**0.07**	<0.010	<0.010
EOX	mg/l	0.01	<0.010	<0.010	**0.019**	<0.010	<0.010
Bromate	µg/l	1.0	<1.0	**1.9**	<1.0	<1.0	<1.0

Table 5 Disinfection by-products analysis in treated seawater in test cycle 9.

Disinfection by-products	Unit	Detection limit	Influent	Treated		Control	
Time (day)			**0**	**0**	**5**	**0**	**5**
Parameter							
Test Cycle 9 (Salinity <22 PSU)							
Trichloromethane (chloroform)	µg/l	0.1	-	<0.10	**0.1**	-	-
Bromodichloromethane	µg/l	0.1	-	<0.10	**0.2**	-	-
Dibromochlorormetane	µg/l	0.1	-	**1**	**4.6**	-	-
Tribromomethane (bromoform)	µg/l	0.1	-	**26**	**120**	-	-
Chloroacetic acid (MCAA)	µg/l	0.5	-	<0.50	<0.50	-	-
Dichloroacetic acid (DCAA)	µg/l	0.3	-	<0.30	<0.30	-	-
Trichloroacetic acid (TCAA)	µg/l	0.2	-	<0.20	<0.20	-	-
Bromoacetic acid (MBAA)	µg/l	0.2	-	<0.20	<0.20	-	-
Dibromoacetic acid (DBAA)	µg/l	0.1	-	**2.4**	**1.1**	-	-
Bromochloroacetic acid (BCAA)	µg/l	0.1	-	<0.10	<0.10	-	-
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	-	<0.10	<0.10	-	-
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	-	<0.10	<0.10	-	-
Tribromoacetic acid (TBAA)	µg/l	0.1	-	<0.10	<0.10	-	-
Dichloroacetonitrile	µg/l	0.1	-	<0.10	<0.10	-	-
Trichloroacetonitrile	µg/l	0.1	-	<0.10	<0.10	-	-
2,4-Dibromophenol	µg/l	0.1	-	<0.10	<0.10	-	-
2,6-Dibromophenol	µg/l	0.1	-	<0.10	<0.10	-	-
2,4,6-Tribromophenol	µg/l	0.1	-	**0.1**	<0.10	-	-
1,2-Dibromoethane	µg/l	0.1	-	<0.10	<0.10	-	-
1,2,4-Tribromobenzene	µg/l	0.1	-	<1.0	<1.0	-	-
1,2,3-Trichloropropane	µg/l	0.1	-	<0.10	<0.10	-	-
2-Chlorotoluene	µg/l	0.1	-	<0.10	<0.10	-	-
4-Chlorotoluene	µg/l	0.1	-	<0.10	<0.10	-	-
1,2-Dibromo-3-chloropropane	µg/l	0.1	-	<0.10	<0.10	-	-
1,2,3-Tribromobenzene	µg/l	0.1	-	<1.0	<1.0	-	-
1,3,5-Tribromobenzene	µg/l	0.1	-	<1.0	<1.0	-	-
Monobromoacetonitrile	µg/l	0.1	-	<0.10	<0.10	-	-
Dibromoacetonitrile	µg/l	0.1	-	<0.10	<0.10	-	-
Bromochloroacetonitrile	µg/l	0.1	-	<0.10	**0.5**	-	-
Dibromomethane	µg/l	0.1	-	**0.2**	**0.4**	-	-
AOX	mg/l	0.01	-	**0.02**	**0.06**	-	-
EOX	mg/l	0.01	-	<0.010	**0.018**	-	-
Bromate	µg/l	1.0	-	**2.4**	**1.9**	-	-

Table 6 Disinfection by-products analysis in treated seawater in test cycle 10.

Disinfection by-products	Unit	Detection limit	Influent	Treated		Control	
Time (day)			**0**	**0**	**5**	**0**	**5**
Parameter							
Test Cycle 10 (Salinity <22 PSU)							
Trichloromethane (chloroform)	µg/l	0.1	-	<0.10	<0.10	-	<0.10
Bromodichloromethane	µg/l	0.1	-	<0.10	**0.2**	-	<0.10
Dibromochlorormetane	µg/l	0.1	-	**1.3**	**4.4**	-	<0.10
Tribromomethane (bromoform)	µg/l	0.1	-	**40**	**130**	-	<0.10
Chloroacetic acid (MCAA)	µg/l	0.5	-	<0.50	<0.50	-	<0.50
Dichloroacetic acid (DCAA)	µg/l	0.3	-	<0.30	<0.30	-	<0.30
Trichloroacetic acid (TCAA)	µg/l	0.2	-	<0.20	<0.20	-	<0.20
Bromoacetic acid (MBAA)	µg/l	0.2	-	<0.20	<0.20	-	<0.20
Dibromoacetic acid (DBAA)	µg/l	0.1	-	**0.95**	**1.6**	-	<0.10
Bromochloroacetic acid (BCAA)	µg/l	0.1	-	<0.10	<0.10	-	<0.10
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	-	<0.10	<0.10	-	<0.10
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	-	<0.10	<0.10	-	<0.10
Tribromoacetic acid (TBAA)	µg/l	0.1	-	<0.10	<0.10	-	<0.10
Dichloroacetonitrile	µg/l	0.1	-	<0.10	<0.10	-	<0.10
Trichloroacetonitrile	µg/l	0.1	-	<0.10	<0.10	-	<0.10
2,4-Dibromophenol	µg/l	0.1	-	<0.10	<0.10	-	<0.10
2,6-Dibromophenol	µg/l	0.1	-	<0.10	<0.10	-	<0.10
2,4,6-Tribromophenol	µg/l	0.1	-	<0.10	**0.1**	-	<0.10
1,2-Dibromoethane	µg/l	0.1	-	<0.10	<0.10	-	<0.10
1,2,4-Tribromobenzene	µg/l	0.1	-	<1.0	<1.0	-	<1.0
1,2,3-Trichloropropane	µg/l	0.1	-	<0.10	<0.10	-	<0.10
2-Chlorotoluene	µg/l	0.1	-	<0.10	<0.10	-	<0.10
4-Chlorotoluene	µg/l	0.1	-	<0.10	<0.10	-	<0.10
1,2-Dibromo-3-chloropropane	µg/l	0.1	-	<0.10	<0.10	-	<0.10
1,2,3-Tribromobenzene	µg/l	0.1	-	<1.0	<1.0	-	<1.0
1,3,5-Tribromobenzene	µg/l	0.1	-	<1.0	<1.0	-	<1.0
Monobromoacetonitrile	µg/l	0.1	-	<0.10	<0.10	-	<0.10
Dibromoacetonitrile	µg/l	0.1	-	**0.5**	<0.10	-	<0.10
Bromochloroacetonitrile	µg/l	0.1	-	<0.10	<0.10	-	<0.10
Dibromomethane	µg/l	0.1	-	**0.3**	**0.4**	-	<0.10
AOX	mg/l	0.01	-	**0.02**	**0.09**	-	**0.05**
EOX	mg/l	0.01	-	<0.010	**0.018**	-	<0.010
Bromate	µg/l	1.0	-	**2.6**	**1.7**	**-**	<1.0

Table 7 Disinfection by-products analysis in treated brackishwater in test cycle 12.

Disinfection by-products	Unit	Detection limit	Influent	Treated		Control	
Time (day)			**0**	**0**	**5**	**0**	**5**
Parameter							
Test Cycle 12 (Salinity <22 PSU)							
Trichloromethane (chloroform)	µg/l	0.1	-	-	<0.10	-	-
Bromodichloromethane	µg/l	0.1	-	-	**0.3**	-	-
Dibromochlorormetane	µg/l	0.1	-	-	**10**	-	-
Tribromomethane (bromoform)	µg/l	0.1	-	-	**410**	-	-
Chloroacetic acid (MCAA)	µg/l	0.5	-	-	<0.50	-	-
Dichloroacetic acid (DCAA)	µg/l	0.3	-	-	<0.30	-	-
Trichloroacetic acid (TCAA)	µg/l	0.2	-	-	<0.20	-	-
Bromoacetic acid (MBAA)	µg/l	0.2	-	-	<0.20	-	-
Dibromoacetic acid (DBAA)	µg/l	0.1	-	-	**0.81**	-	-
Bromochloroacetic acid (BCAA)	µg/l	0.1	-	-	<0.10	-	-
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	-	-	<0.10	-	-
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	-	-	<0.10	-	-
Tribromoacetic acid (TBAA)	µg/l	0.1	-	-	<0.10	-	-
Dichloroacetonitrile	µg/l	0.1	-	-	<0.10	-	-
Trichloroacetonitrile	µg/l	0.1	-	-	<0.10	-	-
2,4-Dibromophenol	µg/l	0.1	-	-	<0.10	-	-
2,6-Dibromophenol	µg/l	0.1	-	-	<0.10	-	-
2,4,6-Tribromophenol	µg/l	0.1	-	-	<0.10	-	-
1,2-Dibromoethane	µg/l	0.1	-	-	<0.10	-	-
1,2,4-Tribromobenzene	µg/l	0.1	-	-	<1.0	-	-
1,2,3-Trichloropropane	µg/l	0.1	-	-	<0.10	-	-
2-Chlorotoluene	µg/l	0.1	-	-	<0.10	-	-
4-Chlorotoluene	µg/l	0.1	-	-	<0.10	-	-
1,2-Dibromo-3-chloropropane	µg/l	0.1	-	-	<0.10	-	-
1,2,3-Tribromobenzene	µg/l	0.1	-	-	<1.0	-	-
1,3,5-Tribromobenzene	µg/l	0.1	-	-	<1.0	-	-
Monobromoacetonitrile	µg/l	0.1	-	-	<0.10	-	-
Dibromoacetonitrile	µg/l	0.1	-	-	<0.10	-	-
Bromochloroacetonitrile	µg/l	0.1	-	-	<0.10	-	-
Dibromomethane	µg/l	0.1	-	-	<0.10	-	-
AOX	mg/l	0.01	-	-	**0.32**	-	-
EOX	mg/l	0.01	-	-	**0.031**	-	-
Bromate	µg/l	1.0	-	-	**6.0**	-	-

Table 8 Disinfection by-products analysis in treated brackishwater in test cycle 13.

Disinfection by-products	Unit	Detection limit	Influent	Treated		Control	
Time (day)			**0**	**0**	**5**	**0**	**5**
Parameter							
Test Cycle 13 (Salinity <22 PSU)							
Trichloromethane (chloroform)	µg/l	0.1	-	-	<0.10	-	-
Bromodichloromethane	µg/l	0.1	-	-	**0.3**	-	-
Dibromochlorormetane	µg/l	0.1	-	-	**9.2**	-	-
Tribromomethane (bromoform)	µg/l	0.1	-	-	**330**	-	-
Chloroacetic acid (MCAA)	µg/l	0.5	-	-	<0.50	-	-
Dichloroacetic acid (DCAA)	µg/l	0.3	-	-	<0.30	-	-
Trichloroacetic acid (TCAA)	µg/l	0.2	-	-	<0.20	-	-
Bromoacetic acid (MBAA)	µg/l	0.2	-	-	<0.20	-	-
Dibromoacetic acid (DBAA)	µg/l	0.1	-	-	**1.8**	-	-
Bromochloroacetic acid (BCAA)	µg/l	0.1	-	-	<0.10	-	-
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	-	-	**0.13**	-	-
Dibromochloroacetic acid (DBCAA)	µg/l	0.1	-	-	<0.10	-	-
Tribromoacetic acid (TBAA)	µg/l	0.1	-	-	<0.10	-	-
Dichloroacetonitrile	µg/l	0.1	-	-	<0.10	-	-
Trichloroacetonitrile	µg/l	0.1	-	-	<0.10	-	-
2,4-Dibromophenol	µg/l	0.1	-	-	<0.10	-	-
2,6-Dibromophenol	µg/l	0.1	-	-	<0.10	-	-
2,4,6-Tribromophenol	µg/l	0.1	-	-	<0.10	-	-
1,2-Dibromoethane	µg/l	0.1	-	-	<0.10	-	-
1,2,4-Tribromobenzene	µg/l	0.1	-	-	<1.0	-	-
1,2,3-Trichloropropane	µg/l	0.1	-	-	<0.10	-	-
2-Chlorotoluene	µg/l	0.1	-	-	<0.10	-	-
4-Chlorotoluene	µg/l	0.1	-	-	<0.10	-	-
1,2-Dibromo-3-chloropropane	µg/l	0.1	-	-	<0.10	-	-
1,2,3-Tribromobenzene	µg/l	0.1	-	-	<1.0	-	-
1,3,5-Tribromobenzene	µg/l	0.1	-	-	<1.0	-	-
Monobromoacetonitrile	µg/l	0.1	-	-	<0.10	-	-
Dibromoacetonitrile	µg/l	0.1	-	-	<0.10	-	-
Bromochloroacetonitrile	µg/l	0.1	-	-	<0.10	-	-
Dibromomethane	µg/l	0.1	-	-	**0.8**	-	-
AOX	mg/l	0.01	-	-	**0.13**	-	-
EOX	mg/l	0.01	-	-	**0.028**	-	-
Bromate	µg/l	1.0	-	-	**4.0**	-	-

Appendix 2 – Chemical fate analysis of disinfection by-products

Table 1 Chemical fate analysis of disinfection by-products in treated and control brackish water in test cycle 5.

Disinfection by-products by ALS Scandinavia	Unit	Detection limit	Influent	Treated water								Control water	
Test Cycle 5 (Salinity <22 PSU) Chemical fate analysis				After first treatment		After second treatment						After second by-pass	
Time (day, hours)			0	day 0	day 5	0h	0,5h	2h	4h	24h	48h	0	48h
Parameter													
Trichloromethane (chloroform)	µg/l	0.1	-	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	<0.10	<0.10
Bromodichloromethane	µg/l	0.1	-	0.4	0.8	0.8	0.8	0.7	0.7	0.7	0.7	<0.10	<0.10
Dibromochloromethane	µg/l	0.1	-	7.4	19	18	18	18	18	18	18	<0.10	<0.10
Tribromomethane (bromoform)	µg/l	0.1	-	190	660	670	680	680	650	650	660	<0.10	<0.10
Dibromoacetic acid (DBAA)	µg/l	0.1	-	12	3.2	3.1	4.4	9.8	6.9	7.6	7.9	0.1	0.10
Bromochloroacetic acid (BCAA)	µg/l	0.1	-	0.41	0.1	0.18	0.18	0.7	0.32	0.37	0.33	<0.10	<0.10
Monobromoacetonitrile	µg/l	0.1	-	<0.10	<0.10	<0.10	<0.10	<0.10	<1.0	<1.0	<1.0	<0.10	<1.0
Dibromomethane	µg/l	0.1	-	<0.10	1.4	<0.10	<0.10	<0.10	1.3	1.1	1.1	<0.10	<0.10
AOX	mg/l	0.01	-	0.08	0.15	0.14	0.16	0.18	0.14	0.16	0.16	<0.010	<0.010
EOX	mg/l	0.01	-	0.02	0.049	0.045	0.044	0.046	0.058	0.041	0.04	<0.010	<0.010
Bromate	µg/l	1.0	-	7.2	9.1	6.6	10	11	11	11	11	<1.0	<1.0

Table 2 Chemical fate analysis of disinfection by-products in treated and control seawater in test cycle 10.

Disinfection by-products by ALS Scandinavia	Unit	Detection limit	Influent	Treated water									Control water	
Test Cycle 10 (Salinity >32 PSU) Chemical fate analysis				After first treatment			After second treatment						After second by-pass	
Time (day, hours)			0	day 0	day 2	day 5	0h	0,5h	2h	4h	24h	48h	0	48h
Parameter														
Bromodichloromethane	µg/l	0.1	-	<0.10	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	<0.10	<0.10
Dibromochloromethane	µg/l	0.1	-	1.3	3.9	4.6	4.4	4.7	4.7	5	5.2	4.9	<0.10	<0.10
Tribromomethane (bromoform)	µg/l	0.1	-	40	130	160	130	160	160	170	190	180	<0.10	<0.10
Dibromoacetic acid (DBAA)	µg/l	0.1	-	0.95	0.47	0.1	1.6	<0.10	0.55	1.7	4.9	2.3	<0.10	0.19
Dichlorobromoacetic acid (DCBAA)	µg/l	0.1	-	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.32	0.14	<0.10	<0.10
2,4,6-Tribromphenol	µg/l	0.1	-	<0.10	<0.10	<0.10	0.1	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromoacetonitrile	µg/l	0.1	-	0.5	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Dibromomethane	µg/l	0.1	-	0.3	0.4	0.5	0.4	0.5	0.4	0.5	0.5	0.4	<0.10	<0.10
AOX	mg/l	0.01	-	0.02	0.13	0.06	0.09	0.11	0.06	0.1	0.08	0.09	0.05	0.05
EOX	mg/l	0.01	-	<0.010	0.015	0.017	0.018	0.017	0.017	0.018	0.017	0.016	<0.010	<0.010
Bromate	µg/l	1.0	-	2.6	2.5	1.9	1.7	2.2	2.4	2.5	2.5	2.6	<1.0	<1.0

Appendix 3 – Detailed inventory of species in the ≥10-50 µm group observed in the dilution cultures and their MPN estimate for treated samples

Organism	size	Cycle 1		Cycle 2*		Cycle 3		Cycle 4		Cycle 5	
	µm	day 0	day 5	day 0	day 5	day 0	day 5	day 0	day 5	day 0	day 5
Small dinofl.	12	5	1.3	50	24	30	8	13	1.7	14	1.3
Small dinofl.	15					1.1					0.2
Alexandrium sp	30	0.2				1.1		1.4	0.8	0.7	
Prorocentrum micans	20	0.2		0.4	0.2	1.8			0.7		
Gyrodinium aureolum	20	0.2		0.4							
Ciliates	15		0.2						0.4		0.2
Tetraselmis	8-11		3		0.2		0.2				
Cryptomonas	9-12			2.3		1.1			8		
sum >10 µm		5.6	4.5	53.1	24.4	35.1	8.2	14.4	11.6	14.7	1.7

*Technical failure

In addition to the algae species shown in the table above, viable algal species with minimum diameter less than 10 µm were found in the test cycles 1 to 5. The most numerous species was *Emiliania huxleyi*. By microscopy inspection of seawater samples at Solbergstrand, a size range of 4.5-6 µm in minimum diameter was determined for this algae.

In test cycle 4 it was observed 8 individual cells of the group Cryptophycea which were determined to be in the size range 9-12 µm. Tests at NIVA of an growing culture and one stationary phase culture of *Cryptomonas baltic* shows different size distribution profiles of this organism. Depending on wether one or the other size distribution profile is dominant in the treated water, 0, 1 or 2 cells of this organism will be found to be below 10 µm and the total count will then be 9.6, 10.6 or 11.6 organisms per ml. In the interpretation of the data, it must be taken into account the limitations of the methodologies with regards to precision. This adds uncertainty to the values which also have to be taken into consideration when the organism counts are close to the IMO requirement concentrations.

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